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To all the people who believe in me
and help me accomplish my goals.

TABLE OF CONTENTS

ABBREVIATIONS

Ab	Antibody	**MSN(s)**	Mesoporous silica nanoparticle(s)
ADME(T)	Absorption, distribution, metabolism, elimination, (toxicity)	**NE(s)**	Nanoemulsion(s)
Ag NP(s)	Silver nanoparticle(s)	**NIR**	Near-infrared
API(s)	Active Pharmaceutical Ingredient(s)	**NLC(s)**	Nanostructured lipid carrier(s)
ATRA	All-trans retinoic acid	**o/w**	Oil-in-water
AUC	Area Under the Curve	**Pah7/Tyr**	Human tyrosinase plasmid
CD	Cyclodextrin	**PAMAM**	Poly (amidoamine)
CMC	Critical micelle concentration	**PBS**	Phosphate buffered saline
CTAB	Cetyltrimethylammonium bromide	**PC**	Phosphatidyl choline
DCP	Dicetyl phosphate	**PCL**	Poly (caprolactone)
DDAB$_{18}$	Dioctadecyl dimethyl ammonium bromide	**PDPA**	Poly [2-(diisopropylamino)ethyl methacrylate]
DDAK	Dodecyl 6-(dimethylamino) hexanoate	**PE(s)**	Penetration enhancer(s)
DMPA	Dimyristoyl phosphatidic acid	**PEG**	Poly (ethylene glycol)
DMPC	Dimyristoyl phosphatidyl choline	**PEG8DL**	Poly (ethylene glycol-8-dilauryl ester)
DMPS	Dimyristoyl phosphatidyl serine	**PEG8L**	Poly (ethylene glycol-8-lauryl ester)
DMSO	Dimethyl sulfoxide	**PEO**	Poly (ethylene oxide)
DOPC	Dioleoyl phosphatidyl choline	**PLA**	Poly (lactic acid)
DOPG	Dioleoyl-sn-glycero-3-phospho-(1'-rac-glycerol)	**PLGA**	Poly (lactic-co-glycolic acid)
DOTAP	Dioleoyl-3-trimethylammonium-propane	**PLL**	Poly-L-lysine
DPPC	Dipalmitoyl phosphatidyl choline	**PMPC**	Poly (2-methacryloyloxyethyl phosphorylcholine)
DSC	Differential scanning calorimetry	**PPO**	Poly (phenylene oxide)
EE	Entrapment/Encapsulation efficiency	**PVA**	Poly (vinyl alcohol)
EPC	Egg phosphatidyl choline	**PVP**	Poly (vinyl pyrrolidone)
FA	Fatty Acid	**QD(s)**	Quantum dot(s)
Fe NP(s)	Iron nanoparticle(s)	**r-GO**	reduced Graphene oxide
FTIR	Fourier-transform infrared spectroscopy	**R-PMPDAP**	9-[2-(R)-(phosphonomethoxy)propyl]-2,6-diaminopurine
GNP(s)	Gold nanoparticle(s)	**SA**	Stearyl amine
GO	Graphene oxide	**SC**	Stratum corneum
GRAS	Generally recognized as safe	**SDS**	Sodium dodecyl sulfate
HA	Hyaluronic acid	**SEM**	Scanning electron microscopy
HLB	Hydrophilic lipophilic balance	**SLN(s)**	Solid lipid nanoparticle(s)
HPMC	Hydroxypropyl methylcellulose	**SPC**	Soybean phosphatidyl choline
HSPC	Hydrogenated soybean phosphatidyl choline	**SUV(s)**	Small unilamellar vesicle(s)
IRF3	Interferon regulatory factor 3	**TAT**	Transactivator of transcription
L-DOPA	Levodopa	**TEM**	Transmission electron microscopy
LNP(s)	Lipid nanoparticle(s)	**Tm**	Melting point
LUV(s)	Large unilamellar vesicle(s)	**UV(s)**	Unilamellar vesicle(s)
MEL	Mannosylerythritol lipid	**w/o**	Water-in-oil
MLV(s)	Multilamellar vesicle(s)		

ABSTRACT

Transdermal administration has gained much attention over the last years due to the remarkable advantages such as patient compliance, drug escape from first-pass elimination, favorable pharmacokinetic profile and prolonged release properties. However, the major limitation of these systems is the limited skin penetration of the stratum corneum, the skin's most important barrier, which protects the body from the insertion of substances from the outer environment. Transdermal drug delivery systems are aiming to the disruption/fluidization of the stratum corneum in order for the active pharmaceutical ingredients to enter successfully the systemic circulation. Therefore, nanoparticles are holding a great promise because they can act as effective penetration enhancers due to their small size and other physicochemical properties that will be analyzed thoroughly in this report. Apart from the investigation of the physicochemical parameters, a comparison between the different types of nanoparticles will be performed. It is highlighted that the complexity of skin anatomy and the unclear mechanisms of skin penetration should be taken into consideration in order to reach some realistic conclusions regarding the way that the described parameters affect the skin permeability. To the best of the authors knowledge, this is the first report on the literature describing the technology of transdermal delivery systems and how this technology affects the biological activity.

Keywords: transdermal, drug delivery, stratum corneum, skin penetration, nanoparticles, physicochemical properties

A. INTRODUCTION

The skin, considered as a multilayer interface between the body and the external environment, is the largest organ in the body (15% of the total body mass) with a primary protective role[1–5]. Skin anatomy is divided in three layers: the epidermis, the outermost layer of skin, consisting mainly of keratinocytes, cells which provide a waterproof barrier, the dermis, the middle layer, that consists of collagen, sweat glands and hair follicles and the hypodermis, the deeper layer, including connective tissue, fat and vessels (mainly blood)[3]. The outermost layer of the epidermis is called stratum corneum (SC) and consists of specific flattened cells (corneocytes) that act mainly as a physical barrier[1,6,7]. SC is composed of proteins (75-80%), intercellular lipids (5-15%) and other components (5-10%). Intercellular lipids include mostly ceramides, as well as cholesterol and fatty acids[7]. The role of SC is double, as it prevents the entering of external "unwanted" substances, but also prevents the body from dehydration[8]. The organization of SC is described in a "brick and mortar" model because the corneocytes, act as bricks, and the lipids resemble to the mortar in a brick wall[5,9].

The main pathways for the entry of substances inside the skin layers are three. Firstly, the intercellular route which is mainly for lipophilic molecules possessing a small size, that pass between the corneocytes of the SC. Secondly, the transcellular route, transportation through corneocytes, which is very selective and mainly for polar or hydrophilic molecules. Lastly, the trans-appendageal pathway (via sebaceous glands, sweat glands and hair follicles) is mainly for water-soluble substances and in this case the appendages act as local-reservoirs[2,10]. This pathway up to date was not considered as significant in drug diffusion as appendages cover only a small portion of the total surface (0.1%)[9].

B. THEORY

Dermal or topical administration of API's is considered their skin delivery, mainly in pathological sites, exhibiting local actions and avoiding any systemic absorption. Target sites include the nerves, keratinocytes, melanocytes, hair follicles and Langerhans cells in the viable epidermis. On the other hand, transdermal administration is the drug diffusion in deeper skin layers until the API reaches the systemic circulation. In this type of drug delivery, the API penetrates the epidermis and reaches the dermis which is supplied with blood vessels[11–14].

Transdermal drug delivery offers several unique advantages over oral and intravenous delivery systems and for this reason a growing tendency in this area has been observed in the last years. Firstly, transdermal delivery of drugs is a painless method with an excellent patient compliance. Besides, even self-administration of drugs or vaccines can be possible via this approach. Moreover, the first-pass metabolism of drug is avoided and thus a better drug bioavailability can be achieved. Also, the duration of drug's action can be increased, and the dose frequency could be reduced, resulting thus, in the decrease of side-effects. Therefore, an improved absorption, distribution, metabolism, elimination and toxicity (ADME(T)) profile can be achieved. Overall, a better dermatokinetic profile in terms of prolonged drug release is suggested, as skin can act as a local depot[15–18].

However, the main challenge about these systems that needs to be overpassed, is their intrinsic difficulty in skin penetration. The "stiff" nature of SC acts as a barrier and restricts the majority of drugs from entering the skin. Another concern regarding transdermal systems is that there is a possibility when they are applied to the skin, to induce a local irritation[15–17]. Generally, the requirements for a successful and safe transdermal delivery of a drug is a molecular weight below 4kDa, with an enhanced lipophilicity, a low melting point and an adequate polarity. The afore-mentioned

specific physicochemical properties render suitable only a limited number of drugs to be tested for transdermal delivery, as most of them do not fulfill these criteria[19–21].

In this point, nanoparticles can offer many advantages, overcoming the limitations of transdermal drug delivery and offering new possibilities. The mechanisms of nanoparticles' skin penetration are shown in Figure 1. Analytically, with NPs a deeper skin penetration can be achieved as they act as penetration enhancers by disrupting the skin barrier. Also, NPs can prolong the drug release and thus significantly reduce the dose frequency and the side-effects. Furthermore, they can be carriers for both hydrophilic and hydrophobic compounds as well as for larger molecules. The improved efficacy and pharmacological activity should be also given to the skin adhesion of NPs and their proximity with the target cells[13,22–24]. However, this new area of nanoparticle-based transdermal carriers should be investigated carefully, as a risk evaluation strategy is necessary before the clinical translation of these formulations in order to avoid long and short-term nanotoxicity effects.
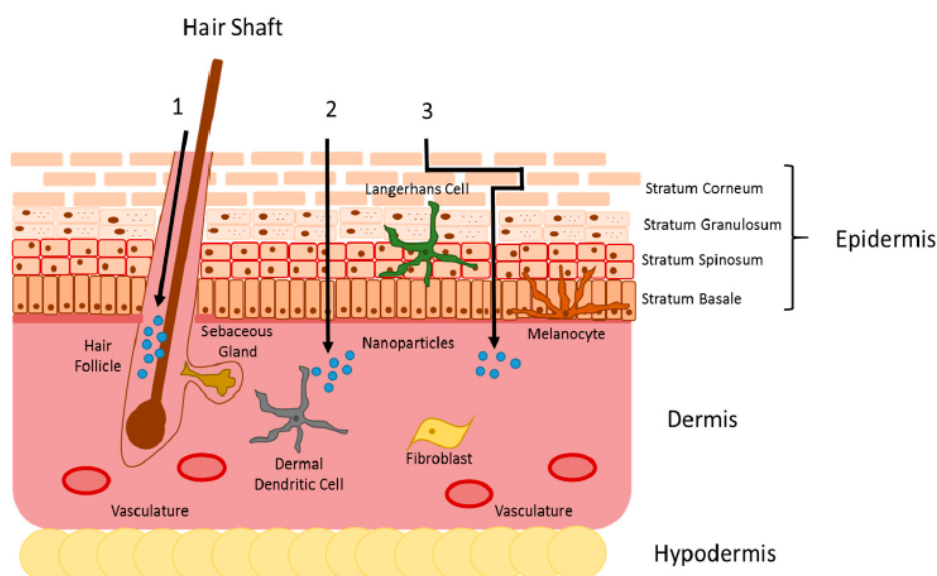

Figure 1. Illustration of nanoparticle skin penetration pathways. Topically applied nanoparticles can penetrate the skin in one of three different ways: (**1**) through the appendageal route, (**2**) through the intracellular route, or (**3**) through the intercellular route. The appendageal route involves nanoparticles entering hair follicles, sweat glands, or skin furrows for either penetration to the dermis or retention for increased drug release. The intracellular route involves a direct path through the cell membrane of

multiple layers of the epidermis. The intercellular route involves a more tortuous path between epidermal cells. The pathway taken likely depends on the nanoparticle size, charge, morphology, and material. (Adapted from [22]).

The aim of the present investigation is to give a detailed analysis of all the different types of nanoparticles used in transdermal drug delivery systems as well as to present the physicochemical properties affecting the effectiveness of this type of administration. It is of high importance to be stated that the interplay of all physicochemical factors affects the skin penetration of NPs and the final efficacy of the systems. Therefore, every single parameter cannot be studied separately due to the intrinsic complexity of the system.

C. METHODS

Systematic search and review of papers and book chapters regarding transdermal administration of different types of nanoparticles (i.e. liposomes, ethosomes, micelles, etc.) took place via MedLine, Scopus, Web of Science platforms, and abstract presentations of international conferences. The papers were chosen after a thorough screening process and only those that met the inclusion-exclusion criteria were selected for analysis. Also, during the process of selection, the Boolean operators were used in order to narrow down the amount of results.

D. RESULTS-DISCUSSION

1. LIPID-BASED NANOSYSTEMS

The first category of transdermal drug delivery systems that will be analyzed focuses on the liposomal formulations. Liposomes are the first clinically approved nanomedicine. They are characterized as vesicular structures consisting of a lipidic bilayer and an aqueous core. They are composed of natural phospholipids and cholesterol, which acts as a bilayer's fluidity modulator[25,26]. Liposomes were first reported as skin drug delivery carriers for triamcinolone acetonide in 1980 by Mezei and Gulasekharam[27]. Two years later the same authors used the liposomal carrier incorporated into a hydrocolloid gel achieving a higher dermal and epidermal concentration of drug[28]. The above reports proved that liposomal carriers are promising for skin delivery and paved the way for the discovery of new, innovative skin formulations. Among liposomes' advantages are the biocompatibility, biodegradability, enhanced drug's protection, and sustained/controlled release. Thus, a better drug stability and efficacy can be achieved, as the toxicity and side effects are reduced. The entrapment of both hydrophilic and hydrophobic drugs, the controllability of liposome's size and their feasibility for modification are also of high importance[29,30]. Concerning the transdermal administration, the most important advantage of these formulations is their similarity to the lipid composition of stratum corneum (SC) which allows them to penetrate deeper in the epidermis for a better drug absorption and final efficacy[31,32].

Generally, the mechanism of liposomes' interaction with the lipid membrane is still under investigation and highly dependent on the composition and the physicochemical characteristics (size, charge, lamellarity, fluidity) as well as the experimental techniques that have been used for their preparation[33]. Firstly, a potential mechanism is a free-drug action via a direct drug exchange between the liposome and the skin surface where drug permeates skin via intercellular or transcellular pathway. Additionally, liposomes can act as penetration enhancers and after their break down, they enable phospholipids to permeate the SC and thus help

drug to penetrate skin barrier. They can also adhere to skin and fuse with the lipidic environment of SC and then a drug diffusion through skin layers will take place. Lastly, they may penetrate intact the SC through appendageal pathways achieving an enhanced transdermal delivery[34–36]. The afore-mentioned mechanisms are depicted in Figure 2. It has been observed that liposomes show mostly a penetration-enhancing mechanism and are retained in the upper layers of skin in contrast to deformable liposomes, which will be analyzed below, and can achieve deeper penetration[37].
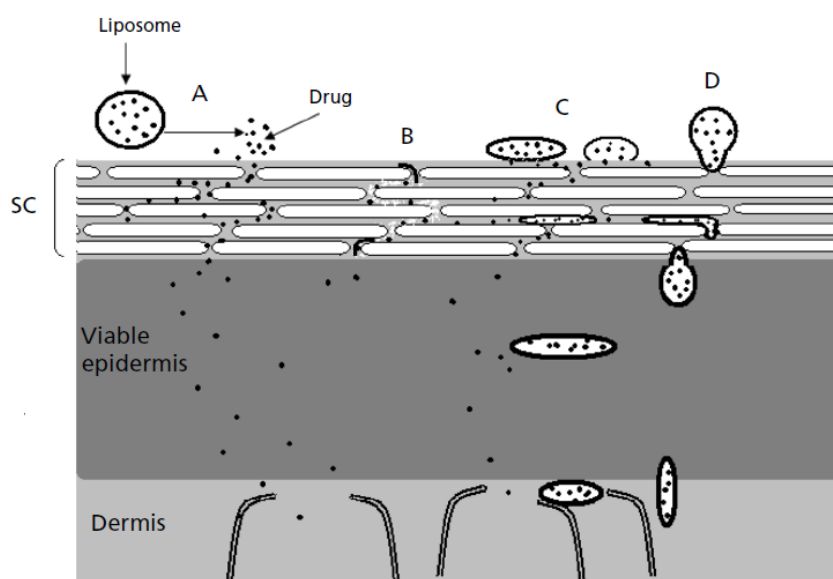

Figure 2. Possible mechanisms of action of liposomes as skin drug delivery systems. **A.** Drug free mechanism. **B.** Penetration enhancing process of liposome components. **C.** Vesicle adsorption to and/or fusion with the stratum corneum (SC). **D.** Intact vesicle penetration into or into and through the intact skin (not to scale). (Adapted from [36]).

For the transdermal administration, liposomes are divided in two main categories: the conventional and the deformable ones. Deformable liposomes (niosomes, ethosomes, invasomes, transferosomes[1]) that will be analyzed below show enhanced flexible properties[38,39]. For aiming a transdermal drug delivery, both vesicles' deformability and transcutaneous hydration should be taken into account[40].

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[1] Both terms transferosome(s) and transfersome(s) are accepted in the literature. However, in this review the term transferosome(s) will be used.

Although liposomal formulations seem very promising for transdermal drug delivery, their low physical stability, tendency for aggregation, the high cost of production and the lack of reproducibility limit their effective scale-up[41,42].

1.1 Liposomes

Liposomes will be thoroughly studied according mainly to their physicochemical characteristics. Among the different reports, cited below, it will be explored how the final effectiveness of these transdermal drug delivery systems is affected by each parameter.

SC is composed of various lipid classes: ceramides, cholesterol, fatty acid (FA) and cholesteryl sulfate, whereas phospholipids are not present. The crystalline phases are predominant along with a minority of lipids in a liquid phase and thus the sandwich model has been proposed, based on the coexistence of both crystalline and liquid phases. Mixtures containing isolated SC lipids mimic SC properties showing that proteins are not of high importance in the skin barrier[43].

Liposomal formulations used for the transdermal route of administration are considered to be in the liquid crystalline state of matter so as to be more flexible, in order to overpass the skin barrier and reach the systemic circulation, in contrast to the topical formulations where the gel state and thus the more rigid structures are preferred[37,38,44]. Thermodynamic state of lipid membrane is of high importance as liquid state vesicles penetrate deeper the skin than gel-state liposomes[45–47]. A deeper penetration of sodium fluorescein with fluid phosphatidyl choline (PC) than rigid hydrogenated PC was reported, whereas cholesterol didn't have any effect[48].

Vázquez-González et al., studied the mixing of lipid monolayers in terms of thermodynamic stability. The system PC:cholesterol:ceramide (0.36:0.24:0.40) was found the best formulation due to the lowest Gibbs energy that was achieved. It should be noted, that PC was used although it is not present in the SC as it is a

component of lamellar bodies which can be degraded by enzymes of SC and result in free FA. Besides, only PC can form liposomes, as FA form only micelles[49].

Various studies have been performed regarding the effect of size in skin permeation. Initially, studies suggested the hypothesis of intact penetration of liposomes below 0.7μm due to their presence in dermis. Intact liposomes were firstly absorbed and then permeated the skin[50]. Esposito et al. reported that the lower the liposomal size, the better the skin permeability[51]. In another study, the highest depot effect and deeper penetration of intermediate particles around 300nm was reported and the assumption regarding skin penetration of intact liposomes was rejected[52]. Kotla et al. reported that above 600nm lipid-based structures could not permeate deeper skin layers in comparison to those below 300nm, that were found to deliver the drug in deeper skin compartments[53]. A comparison between different vesicle sizes showed best results in skin permeability for an optimal size of 120 nm, proving that the penetration is inversely proportional to the size. More specifically, vesicles bigger than 600 nm remain in the SC and they reinforce the skin barrier's properties. Vesicles around 70-300 nm can penetrate the skin in a sufficient way, but sizes below 70 nm are the most effective for enhanced skin permeation[54]. Although, the role of size has not yet been fully understood, it is possible that intact penetration may be a mechanism only for diseased skin[45].

Lamellarity and size are closely related to the skin permeation of a vesicular structure. El Maghraby et al. compared small unilamellar vesicles (SUVs) of 136nm and large unilamellar vesicles (LUVs) of 557 nm assuming that only the first ones could penetrate intact skin. However, the flux values from the in vitro studies were found similar[55]. The idea behind multilamellar vesicles (MLVs) is that they can offer a strong depot effect and thus achieve a prolonged drug release and protect the drug from early clearance or enzymatic reactions. MLVs before their skin permeation, they can be absorbed intact and then unilamellar and oligolamellar vesicles may be formed due to the gradual destruction, the loss of bilayers, of MLV during their penetration[45,50]. MLVs generally are preferred for the transportation of lipophilic drugs due to high lipid

surface in comparison with LUVs that are used for the encapsulation of hydrophilic drugs due to the more aqueous interior[56]. Another comparison between MLVs and unilamellar vesicles (UVs) showed similar results of penetration ability, stating that intact penetration cannot take place. Besides, it was reported that skin penetration was not affected neither by size nor by lamellarity, but only by charge, which will be analyzed above[57].

The presence of charge is of high importance in liposomes as it enhances the repulsive forces between vesicles and thus aggregation phenomena and drug leakage are reduced and better stability can be achieved. Charged phospholipids, several coatings or other charged additives in the bilayer can form charged liposomes[58].

The most common coating that is used is chitosan and its derivatives, providing a positive z-potential to the vesicles, as mentioned above, through the mechanism of charge inversion[59]. Anionic lipids as dioleoyl phosphatidyl choline (DOPC) and dioleoyl-sn-glycero-3-phospho-(1'-rac-glycerol) (DOPG) can enhance the transcutaneous delivery of drugs dispersions under electroporation[60]. Also, anionic lipid dimyristoyl phosphatidyl serine (DMPS) enhanced insulin's delivery into skin, acting synergistically with electroosmosis technique[61]. Furthermore, charged additives dioleoyl-3-trimethylammonium-propane (DOTAP), stearyl amine (SA) and dicetyl phosphate (DCP) enhance the transdermal delivery. Specifically, retinoic acid was successfully delivered transdermally with the addition of DOTAP in liposomes due to the positive charge and its electrostatic attraction with the negatively charged SC[62]. On the other hand, Puglia et al. proved that neutral and negatively charged DCP-liposomes achieved a better sustained profile than the positively charged ones containing dioctadecyl dimethyl ammonium bromide (DDAB$_{18}$)[63]. Sumatriptan succinate was encapsulated in SA and DCP-coated liposomes. With positively charged unilamellar liposomes, the highest loading efficiency was achieved maybe due to a disattachement of SA from the lipidic bilayer because of drug's stronger interactions. For negatively-charged liposomes, drug encapsulation is highly dependent on the ionization degree[56]. DMPA and DCP-liposomes improved skin permeation of betamethasone and betamethasone dipropionate compared to the positively charged

containing SA. Better skin permeation was found for betamethasone as liposomes improved its intrinsic low penetration ability compared to betamethasone propionate which is more lipophilic and possesses an adequate intrinsic permeability[44]. The above results are in agreement with Sinico et al. findings who reported that negatively charged liposomes enhanced retention of Tretinoin and skin hydration[57].

By using charged liposomes, a higher flux was achieved for methyl nicotinate than the neutral ones. Nevertheless, in viscosized polymeric formulations, neutral liposomes resulted in a better flux. In this study, the main finding supports that formulation's viscosity plays a key role in the determining step of drug's absorption, rather than size or charge[51]. L-ascorbic acid-2 phosphate was entrapped in positively and negatively charged MLVs with the addition of DOTAP and dimyristoyl phosphatidic acid (DMPA) respectively. Drug flux was improved significantly with cationic MLVs, as the anionic MLVs because of the repulsive forces with the drug, reduced the encapsulation/entrapment efficiency (EE%). Also, a physical mixture of drug with empty liposomes was compared with L-ascorbic acid-2 phosphate-loaded MLVs and showed better skin permeation, retention and drug protection supporting the main mechanism of cationic MLVs as penetration enhancers, which will be analyzed in depth below[64].

Penetration enhancers (PEs) or edge activators are often connected to high toxicity and irritation due to the alteration of SC properties that sometimes end up being irreversible. The first PE, Azone was patented in 1976[65]. PEs can be classified according to their origin (natural, synthetic, semi-synthetic), to general properties (small molecule solvents, amphiphiles, peptides) and to their chemical class (hydrocarbons, alcohols, amines, carboxylic acids, esters, cyclic and acyclic amides, sulfoxides)[66]. Figure 3. illustrates several possible modes of action for PEs. Firstly, the mechanism that has been proposed for PEs action is described by the lipid-protein partitioning theory. PEs can act either by altering lipid composition of SC and fluidizing the lipid bilayer or by altering keratin's conformation and thus disrupting the cohesion of corneocytes. Also, by modifying the SC nature by creating a PE gradient, the flux can

be increased and a partitioning of drug into the SC can take place. A lot of PEs act by a combination of mechanisms. However, the interactions with lipids of SC are of crucial importance as this is the major pathway for transdermal administration[67,68]. Another theory is the solubility-physicochemical-thermodynamic theory. Solvent properties, physicochemical interactions of PEs with the drug and the skin and thermodynamic activity should be taken into account for effective transdermal formulations[69].
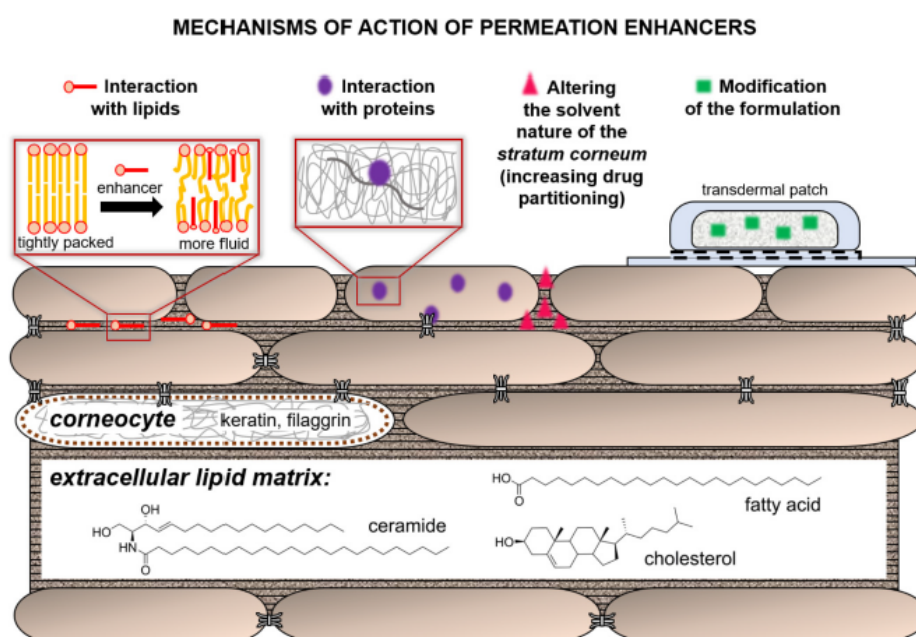

Figure 3. Stratum corneum composition **(A)** and mechanisms of action of permeation enhancers **(B).** (Adapted from [66]).

Another categorization of PEs according to their mechanism of action was the following: Category A (Tween™20, Tween®80, Pluronic®F-127, Pluronic®P-84, FA and derivatives) described by strong disruption of the SC, Category B (Span®40, Span™ 60, Span®80, SDS, hydrophilic non-ionic surfactants with hydrophilic lipophilic balance (HLB) 4-7, anionic surfactants HLB 40) where SC lipid structure was not disrupted and membrane's fluidity was less than the previous category, Category C (Tween™40, Tween™60, Tween®85, Span®20, Brij®58) with similar mechanism of action with category B, but achieving a destruction of SC structure in higher concentrations of PEs, Category D (alcohols, glycols) where PEs interact with hydrophilic regions of the bilayer and Category E (urea and derivatives, pyrrolidone derivatives, ethyl oleate)

with no effect on SC lipids. The mechanism of action of the last category cannot be predicted with the method described in this study. Overall, only the first three categories of non-ionic and anionic surfactants were proved to fluidize the lipid membrane[70].

For safe transdermal formulations, restoration of SC to its normal function is necessary after the use of any formulation for avoidance of side-effects[68]. Mixture of PEs[71] or biodegradable ones have been proposed for minimizing the side effects. For example, cell penetrating peptide-conjugated liposomes displayed a positive zeta potential compared to a negative one for typical liposomes, resulting in enhanced cellular uptake and skin permeation. Cationic-arginine rich peptide strongly interacts with the lipid environment of the SC allowing the effective transdermal administration of *Polygonum aviculare* extract[72]. Also, Salvianolic acid-TAT-liposomes increase the antiproliferative activity of human skin fibroblast cells, dependent upon the concentration and the time. Peptide TAT enhances drug permeation in the skin without any effect on its biological potency. Nevertheless, due to lack of TAT's specificity, poly (ethylene glycol) (PEG) "masking" is necessary to avoid the rapid body clearance[73]. Several 2,6-diaminopurine acyclic nucleoside phosphonate antiviral drugs were studied for their transdermal delivery in the presence of the PE dodecyl 6-(dimethylamino) hexanoate (DDAK). Best results were reported for the anti-human immunodeficiency virus drug ®-PMPDAP. Also, PEG was used as co-enhancer acting synergistically with amphiphilic DDAK[74]. Transcutol as a PE was found to improve membrane's fluidity for the transdermal delivery of both hydrophilic and lipophilic drugs[75]. Specifically, transcutol PE containing vesicles were studied for the transdermal delivery of diclofenac. It was found that these vesicles penetrate intact the SC due to both the synergism between transcutol and vesicles and the interactions with lipids of SC[76]. Lastly, it was found that Tween-20 (edge activator) and d-limonene (PE) act synergistically improving fluorescein sodium penetration[77].

Also, PC acts as a strong PE and its presence is crucial in the vesicular structure. Curcumin-loaded liposomes composed of different phospholipids: soybean phosphatidyl choline (SPC), egg phosphatidyl choline (EPC), hydrogenated soybean

phosphatidyl choline (HSPC) with similar size and EE were compared. Slower release rate, lower skin penetration and drug retention was reported for the hard phospholipid HSPC-formulation due to increased bilayer's rigidity. In contrast, liposomal membrane's fluidity of SPC and EPC-formulations increased the interactions with the cell membrane enhancing the anti-neoplastic activity and drug permeation. SPC-formulation displayed the best results[78]. The mechanism of skin penetration may be totally dependent on PC instead of vesicle's size as expected in the case of transdermal delivery of caffeine[79]. In another study, it was proved that liposomes containing cationic surfactants dimyristoyl phosphatidyl choline (DMPC) and dipalmitoyl phosphatidyl choline (DPPC) instead of EPC, improved skin penetration of retinoic acid due to their interactions with the negatively charged membrane. In this case, the presence of cationic PC is more important than the liposomal composition itself[62]. DMPC contributes significantly to the deformability of liposomes due to the phase transition at 23°C and the liquid crystalline state of matter. Also, the ability of several surfactants was investigated with best results found for poly (ethylene glycol-8-lauryl ester) (PEG8L) and poly (ethylene glycol-8-dilauryl ester) (PEG8DL), exhibiting enhanced fluidity[80]. Esposito et al. reported that methyl nicotinate permeability was enhanced with increased PC concentration. Also, it was found that viscosity plays a crucial role in drug permeability rather than size and charge[51].

Polymer coating improves the skin permeation and stability as generally uncoated liposomes tend to aggregate and result in drug leakage and short circulation time[81]. Chitosan coated liposomes are considered promising carriers for transdermal administration of drugs due to the inversion of z potential to a positive value. For example, better indocyanine green stabilization, skin permeation and enhanced cellular uptake were achieved, all resulting in a more effective photodynamic therapy[82]. Similar results were also reported for the delivery of resveratrol with chitosan-coated liposomes[83]. In another study, the addition of chitosan, decreased the EE% due to the repulsion with the drug (lidocaine), as both possess a positive charge, although better skin permeation and physicochemical stability were reported[84].

Another strategy that is used is the Layer by layer technology, presented in Figure 4., for the formation of multilayer films and more specifically of polyelectrolyte complexes of chitosan[85,86]. Jeon et al. synthesized multilayered liposomes of hyaluronic acid (HA) and chitosan, entrapped in a polymeric gel for the delivery of quercetin. The formed polyelectrolyte complex showed alterations in z-potential, improved the carrier's stability and achieved a sustained drug release. The strong electrostatic interactions between HA and chitosan play a major role as HA due to its hydrophilic nature enhances skin hydration and results in higher stability than chitosan-coating alone. Also, the similar skin permeability for chitosan alone and HA-chitosan liposomes justify that penetration is not dependent absolutely upon the surface charge[87].
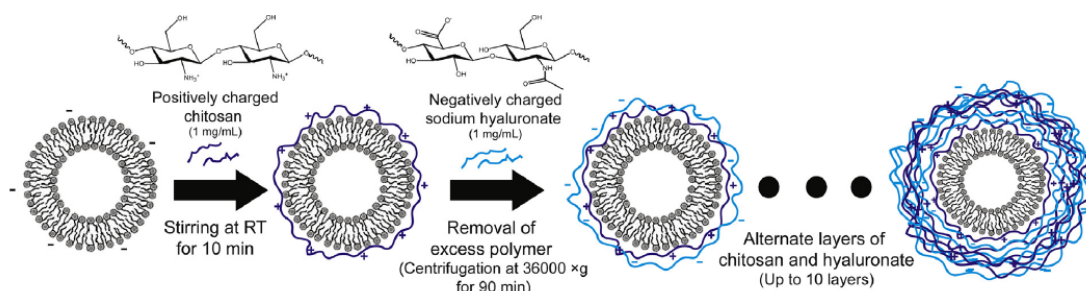

Figure 4. Schematic illustration of the fabrication of multilayered liposomes by Layer-by-Layer deposition of chitosan and sodium hyaluronate. (Adapted from [87]).

Other coatings were also reported for improving transdermal delivery of drugs. A thorough study of the viscoelastic properties of diethylamino-ethyl dextran coated liposomes showed increased gel rigidity resulting in a strong depot effect[88]. High and low-methoxy pectin-coated liposomes showed less aggregation phenomena and lipid oxidation and thus better stability. Also, the decrease in z-potential due to the negative charge of pectin, enhances the skin permeation of Vitamin C[89]. Lastly, it is worth mentioning that PEGylation plays a key role in the transdermal administration, as PEG's bonding with water molecules, hydrates the SC resulting in an enhanced skin permeation. The formed conformational cloud and the added negative z-potential value of PEG-grafted liposomes also improve colloidal stability[90]. Hydrophilic drugs, as fluorescein sodium, were transported transdermally with PEGylated-liposomes which

possessed an enhanced circulation time and stabilization due to the repulsive forces between bilayers[77].

1.2 Deformable liposomes: the main categories

Deformable/elastic liposomes can permeate easily the SC, compared to conventional liposomes achieving enhanced bioavailability, improved systemic effects and prolonged drug release[91,92]. Deformable /elastic liposomes are illustrated in Figure 5. More specifically, invasomes, tranferosomes, ethosomes and niosomes can deliver a variety of drugs transdermally ranging from low molecular weight – Active Pharmaceutical Ingredients (APIs) to macromolecular proteins with a considerable improvement of their efficacy[38]. The general mechanism of these vesicles is mostly their intact transportation through the skin reaching finally the systemic circulation. Xerophobia, the tendency to avoid dry environments, acts as the driving force for the transportation of vesicles, following the hydration gradient. Also, these vesicles can act either as carriers or as vesicles[93,94]. The study of elastomechanic properties of elastic vesicles is of high importance for the design of carriers for biomedical uses[95]. However, there are many reports describing conflicting results of the efficacy of these formulations[96]. Specifically, the main concern is their long-term stability. Nevertheless, a balance between stability and elasticity could be found. For example, in the storage temperature at 25°C, vesicles would exist in a rigid form, but by modifying the Tm of phospholipids, once being applied to the skin at 32°C, they could be designed to exist in a liquid state[97].
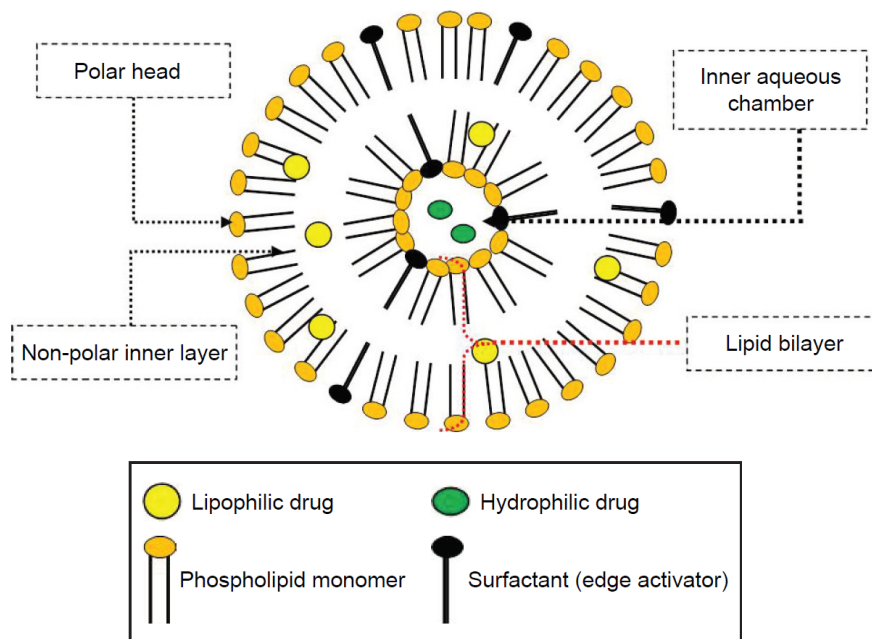
Figure 5. Schematic illustration of bilayer elastic liposomes displaying various components and structural morphology. (Adapted from [96]).

1.2.1 Ethosomes

Ethosomes were reported for the first time by Touitou et al. in 1997 and initially they were patented by Therapeutic Technology Inc. Ethosome[TM]. They are stable formulations, easily prepared, composed of phospholipid, ethanol and water. Several ethosomal formulations are already marketed[35,39]. It is of high importance that the safety of these formulations would be studied thoroughly to avoid skin irritation by ethanol[98].

Ethosomes possess smaller size compared to conventional liposomes, due to the incorporation of increased ethanol concentration, which creates a negative net charge, resulting in a decrease of the liposomal size. Ethosomes increased their size with reducing ethanol concentration between 20-45 %. With ethanol, high EE can be achieved due to enhanced drug solubility, even for lipophilic drugs. Also, ethosomes are found by far more effective than ethanolic solutions, ethanol alone, and ethanolic solutions with phospholipids[99]. Also, the fact that ethosomes' elastic properties are exhibited both in occlusive and non-occlusive conditions should be considered[92,100].

This type of delivery systems has been used for the transdermal administration of a variety of drugs such as testosterone, trihexyphenidyl hydrochloride, even for polypeptides[101].

The mechanism of ethosomes' penetration into the skin is a 2-step mechanism. Firstly, ethanol interacts with the polar head group of SC lipids, lowering their Tm, disturbing the SC organization and thus increasing the fluidity[96]. Ethanol provides also the vesicles with soft flexible characteristics so as to permeate into deeper skin layers via the interlipid pathway. Therefore, alcohol has a double role in these soft vesicles: both the fluidization of the SC lipids and of the lipid bilayers[102]. Then, ethosomes can fuse with skin lipids and release the encapsulated drug along the penetration pathway[41]. Particularly, ethosomes disintegrate in the upper skin layer and phospholipids stay in the upper epidermis in contrast to drugs that penetrate in deeper skin layers[103].

Ethosomes were reported for the transdermal delivery of repaglinide, offering deep penetration, sustained drug release and a better final effectiveness[104]. Similar results were observed for the delivery of macromolecular peptide drugs, like Thymosin β-4[105], for the hydrophilic drug flurbiprofen[106], for tocotrienol used in melanoma therapy[107] and for ropivacaine[108]. Ethosomes were also used for the encapsulation of caffeic acid and a better EE%, stability and antioxidant activity was achieved. Additionally, the entrapment of ethosomes in a Poloxamer 407 gel was studied as a promising formulation technique[109]. Furthermore, entrapment of paeonol in ethosomes exhibited sustained release, increased absorption, and improved skin permeability without any skin irritation, proving the safety of these formulations[110,111]. Ethosomes conjugated with HA, were prepared for the transdermal delivery of the model drug Rhodamine B. It was observed that HA improved the skin permeability due to the formation of a hydration film in ethosomes and the moisturizing effect on the SC. Also, HA added a more negative z-potential value to the ethosomes[112].

Niu et al. through a deep physicochemical characterization of ethosomes, reported a shift of the characteristic peaks from Fourier-transform infrared spectroscopy (FTIR), indicating the disruption of SC's ordered structure. The transition to a disordered state

was also confirmed by the Raman spectroscopy. Additionally, Differential scanning calorimetry (DSC), revealed a shift in Tm due to the fluidization of the phospholipids. From Scanning and Transmittance electron microscopy (SEM, TEM), the microstructure of SC was compared after the application of ethosomes and ethanol alone and it was observed that only in the case of ethanol a swelling of SC and an increase in keratinous space could be seen. Lastly, fluorescence labeling showed that the main pathway for SC penetration was the intercellular route[113].

1.2.2 Transferosomes

Transferosomes, were firstly reported by Cevc and Blume in 1992[94] and comprise the IDEA's proprietary[35]. According to some author, they are considered as the first generation of deformable liposomes[11, 70]. They are composed of phospholipids and surfactants (sodium cholate, sodium deoxycholate, Span 60, Span 65, Span 80, Tween 20, Tween 60, Tween 80 or dipotassium glycyrrhizinate)[71]. These surfactants act as edge activators, by squeezing through the channels of SC, possessing a high radius of curvature and thus disrupt the lipid organization in the SC[31]. The method of preparation as well as the type and amount of surfactants, affect the properties of transferosomes. For example, it was reported that the rotary evaporation method resulted in an increased EE% compared to the vortexing-sonication one. Also, Tween 80 was found the best option for the maximum deformability of vesicles[114].

These channels are about one-tenth of the transferosome size. The basic mechanism for skin penetration is the osmotic gradient due to the different water content in the dehydrated skin surface (20% approximately) and the hydrated environment of epidermis (about 100%). Also, they can act as drug delivery systems, entering intact the SC by modifying their shape and carrying the drug to the skin under the driving force of xerophobia[12,39,91,94]. Transferosomes can be applied either occlusively or non-occlusively. However, due to the osmotic gradient across the skin, which is the driving force for transferosomes' transport, occlusion would eliminate this gradient and as a

result would reduce the transdermal flux, so non-occlusive applications are preferred[94,102].

The encapsulation of pentoxifylline in transferosomes overcomes its low oral bioavailability and limited half-life. The high elasticity of these vesicles given to the edge activator sodium cholate, achieves a prolonged drug release as well as protects vesicles from rupture. The high negative value of zeta potential is crucial for their skin penetration due to the repulsion with the membrane. Also, it is of high importance the flip-flop phenomenon that takes place and increases the half-life of the drug. The carrier mediated mechanism has been described for the transdermal penetration of these vesicles[115]. A comparison between oral tablets and transferosomes regarding the plasma concentration of pentoxifylline is represented in Figure 6. Also, similar results achieved for the asenapine maleate when encapsulated in transferosomes and in the presence of ethanol as a PE which acts synergistically[116]. Stability, bioavailability, solubility and skin permeation were also improved for the transferosomal formulations of resveratrol[117]. Screening techniques were also used for the discovery of the optimal formulation for the transdermal delivery of sildenafil with transferosomes possessing small vesicle size and high EE at the same time. The drug release was found biphasic with a rapid drug release and then a sustained release of the drug[118].
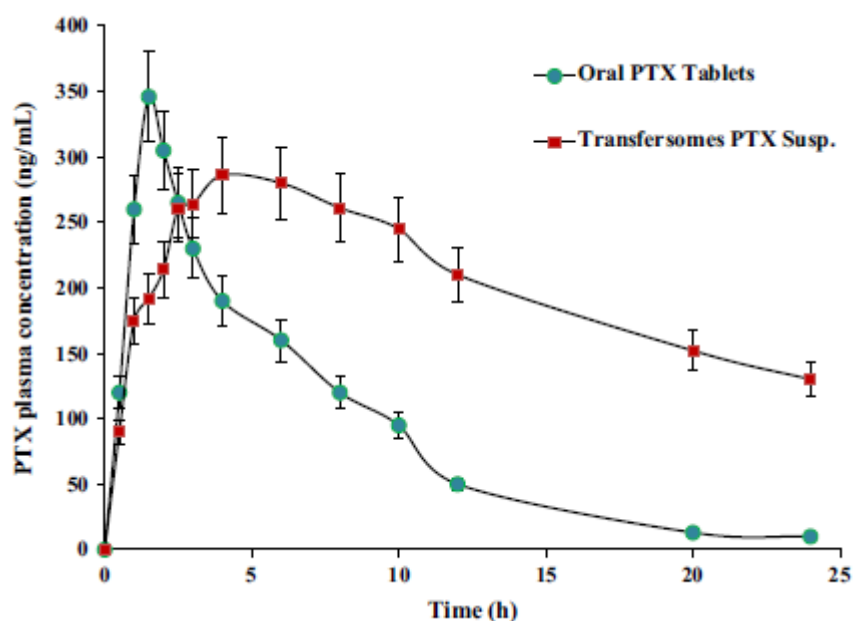
Figure 6. PTX plasma concentration time profile after administration of PTX SR oral tablets and transdermal transfersomes formulation. Data are given as mean ± SD. **Abbreviations:** PTX, pentoxyfilline, SR, sustained-release (Adapted from [115]).

Chaudhary et al. reported that the deformability of transferosomes, depends on lipid and surfactant concentration. For example, high concentration of surfactants reduces the elasticity as micelles are formed instead of vesicles. Similarly, increasing lipid concentration results in more rigid vesicles. Transferosomal formulations containing diclofenac diethyl-amine and curcumin were prepared achieving higher skin penetration, sustained effects and a better bioavailability[119]. Also, emu oil could act as a PE with anti-inflammatory action at the same time. 4-hydroxytamoxifen was encapsulated in transferosomes with and without emu oil for the treatment of breast cancer. No adverse effects were observed and a lower amount of free drug was found in the plasma indicating that there is no off-target distribution. Emu oil is promising for reducing the systemic circulation of the drug and increasing the tumor targeting[120].

1.2.3 Invasomes

Invasomes are composed of PC, ethanol and terpenes. Both ethanol and terpenes act as PEs[39,91]. More specifically, terpenes act by disrupting the tight lipid organization of SC or by disrupting the stacking of bilayers. Several invasomes disintegrate in the upper skin layers and release the phospholipids and terpenes which act as PEs, whereas the smaller ones permeate the skin layers intact. Ethanol, phospholipids and terpenes show synergism in the fluidization of the intercellular lipids[121]. This type of vesicles is very promising as both hydrophilic and lipophilic drugs can be entrapped with an improved skin permeability. However, a balance between potency and toxicity of terpenes should be considered for their safe clinical efficacy[122].

Invasomes containing a mixture of terpenes (cineole, citral and d-limonene) were used for the transportation of the hydrophobic photosensitizer temoporfin used in photodynamic therapy[121]. Also, invasomes containing the terpene β-citronellene were found promising for the delivery of isradipine for the treatment of hypertension with a better final efficacy by the improvement of transdermal flux, of EE but also of the low bioavailability[123,124]. Furthermore, angiotensin receptor II blocker olmesartan medoxomil was entrapped in invasomes with β-citronellene for overcoming its low oral bioavailability. It was found that the drug bioavailability was improved 1.15 times than the oral formulation due to the avoidance of the hepatic metabolism and the achievement of a higher systemic concentration[124,125]. The improvement of the low oral bioavailability with the use of invasomes was also reported for the drug agomelatine. In this report, the combination of active (sonophoresis) and passive (invasomes) permeation techniques was used. For invasomes, 4 terpenes (limonene, cineole, fenchone, citral) were studied in different concentrations, but the best results were reported for limonene. The bioavailability was found 7.29 times higher than the relative Area under the curve (AUC) values. This may be explained by the enhanced lipophilicity of the drug which renders it a good candidate for transdermal delivery as well as the first-pass metabolism avoidance. Key factors are also the penetration enhancement given to invasomes nature and their direct contact with skin due to their high surface area. Finally, sonophoresis plays also a significant role in the

improvement of the skin permeability[126], but it will not be analyzed further as the study of active techniques for skin permeation is out of the scope of this report.

1.2.4 Niosomes

Niosomes are structured by the self-assembly of non-ionic surfactants in an aqueous medium. The addition of non-ionic surfactants, offers enhanced stability and prolonged effects in niosomes, as they can penetrate intact the SC or fuse with the lipid bilayers acting as PEs[35,39]. Firstly, they were patented by L'Oreal in 1975. They can be an alternative to liposomes in terms of cost-effectiveness and stability[127]. They are more stable than conventional liposomes as water loss transepidermally is reduced and lost skin is replaced via the fusion of vesicles with corneocytes[100,128].

Non-ionic surfactants based on polymer materials (Pluronics) and sucrose esters were compared both as niosomal carriers and in the form of sub-micellar formulations, but it was found that only in the vesicular formulations they could act as PEs for sulfadiazine sodium salt transdermal delivery[129]. Transdermal delivery of diacerein, with niosomes composed of Span60, was also reported that overcomes the disadvantages of oral administration and improves patient compliance[130]. Furthermore, several essential oils were investigated for the fluidizing effect of niosomes aiming at the improved transdermal delivery of felodipine. Essential oils act synergistically with niosomes possibly through the diffusion of terpenes which can disrupt the SC structure[131]. Encapsulation of lornoxicam in niosomes enhanced the skin permeation and anti-inflammatory effects, as shown in Figure 7., without any irritation. The gel was characterized by a pseudoplastic behavior as well as a biphasic release profile due to a rapid drug release in the beginning followed by a slower rate[132]. Lastly, niosomes are promising for the transdermal delivery of antihyperlipidemic simvastatin for pediatric applications improving its bioavailability to up to 3-fold compared with the oral drug[133].
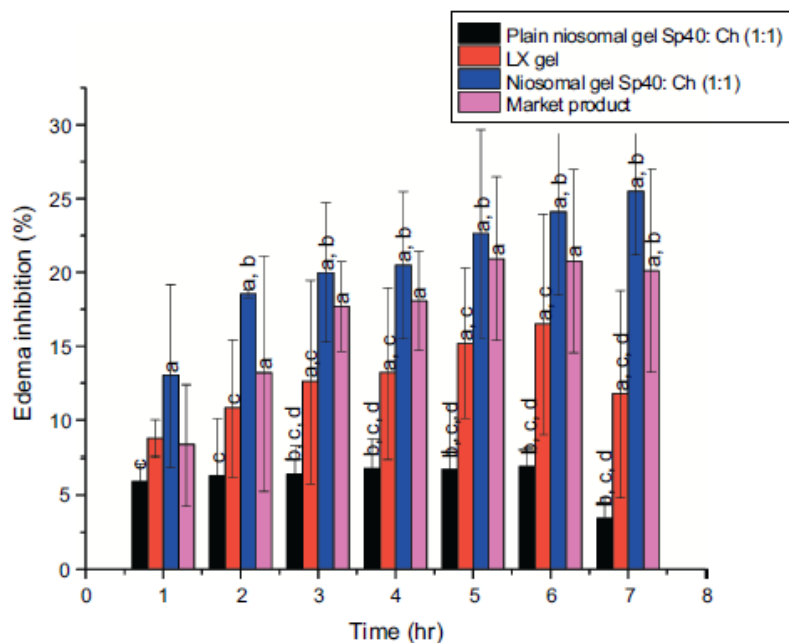
Figure 7. Paw edema inhibition percentages after application of plain niosomal gel, LX gel, LX niosomal gel and market product to carrageenan induced rat paw. "a" significantly different from plain niosomal gel group, "b" significantly different from LX gel group, "c" significantly different from LX loaded niosomal gel group, "d" significantly different from market product, at $P<0.05$. **Abbreviations:** LX, lornoxicam (Adapted from [132]).

Several ways exist for improving vesicles stability, as increasing cholesterol ratio. However, excessive amounts of cholesterol may compete with the drug for encapsulation and finally exclude it. Meloxicam was entrapped in niosomes containing Span-60: cholesterol (6:4) and it was found that the release follows a diffusion-controlled mechanism. Cholesterol amount was decreased in this case as Span-60 possesses a long-saturated alkyl chain. The best drug effectiveness was found for meloxicam niosomal hydrogel than for the gel containing free meloxicam and the commercially available piroxicam gel[134]. Salidroside, a hydrophilic drug with poor skin permeability was entrapped in niosomes composed of Span 40 and cholesterol and showed good biocompatibility and enhanced transdermal flux. Also, the cellular uptake from keratinocytes and fibroblasts showed that niosomes' endocytosis takes place via several channels[135]. Also, salidroside was encapsulated in niosomes containing Span 40, cholesterol and sodium dodecyl sulfate (SDS). The added value of SDS was an increase in niosomes' stability due to a more negative zeta potential. However, excess of SDS caused repulsion between the niosomes and the skin cells due

35

to high negative charge and changed their vesicular structure to a micellar one[136]. Additionally, enhanced transdermal delivery of human tyrosinase plasmid (Pah7/Tyr) was reported with the use of cationic niosomes conjugated with TAT-peptide (PE) holding a great promise for the formation of a future transdermal gene delivery system[137]. Muzzalupo et al. studied also the synergistic effects of surfactants with hydroxyl additives (ethanol, PEG, glycerol). They found that in all cases the vesicle size was increased and an improvement in the percutaneous delivery of sulfadiazine sodium was achieved, but the best results were observed with glycerol. Furthermore, for alcohol concentration up to 40% v/v, the EE was improved, whereas for concentration up to only 20% v/v the drug permeation was increased[138].

Proniosomes are lipid vesicles that after their in-situ hydration from water of the skin surface under occlusive conditions, they are converted in niosomes. Also, instead of water, other hydration solvents like buffers can be used. Proniosomes offer a better stability, shelf life, less aggregation and no leakage phenomena[139]. Proniosomes were used for the encapsulation of risperidone[140]. Mefenamic acid was also encapsulated in Span 80 proniosomal gel for enhanced formulation stability and cholesterol enhanced the drug release and skin permeability[141].

1.3 Other types of liposomes

1.3.1 Bilosomes

Other types of liposomes were reported also as promising formulations for the transdermal delivery of drugs. Specifically, Albash et al. studied PEGylated bilosomes (niosomes combined with bile salts) as carriers for the antihypertensive olmesartan medoxomil with an improved bioavailability as well as a controlled drug release compared to the oral dosage form. The addition of bile salts renders lipid vesicles more stable. Hydrophilic PEG chains help the hydration of the SC and thus the broadening of the intercellular channels[142]. Furthermore, bilosomes were reported for the delivery of ondansetron hydrochloride[143] and tizanidine hydrochloride[144] across the skin, meeting the requirements for safe formulations and overcoming the low oral bioavailability of these drugs. Tenoxicam was also entrapped in bilosomes with a good EE and safety profile[145].

1.3.2 Transethosomes

Transethosomes are reported as the next generation of ethosomes as they are composed of ethanol and surfactants. Sinomenine hydrochloride was intercalated in these vesicles which also possessed an antioxidant surface as they were decorated with ascorbic acid. The antioxidant surface contributed to the ability of transethosomes to target inflamed joints[146]. Figure 8. presents the design and the development of transethosomes in detail. Olmesartan medoxomil was encapsulated in transethosomes and an improved bioavailability was observed without any irritation effect. Also, better deformable properties were observed compared to transferosomes[147]. Poor water solubility and high volatility of the drug paeonol were overcame by its entrapment in transethosomes. It was reported that transethosomes offered a higher EE and AUC compared to transferosomes[148].
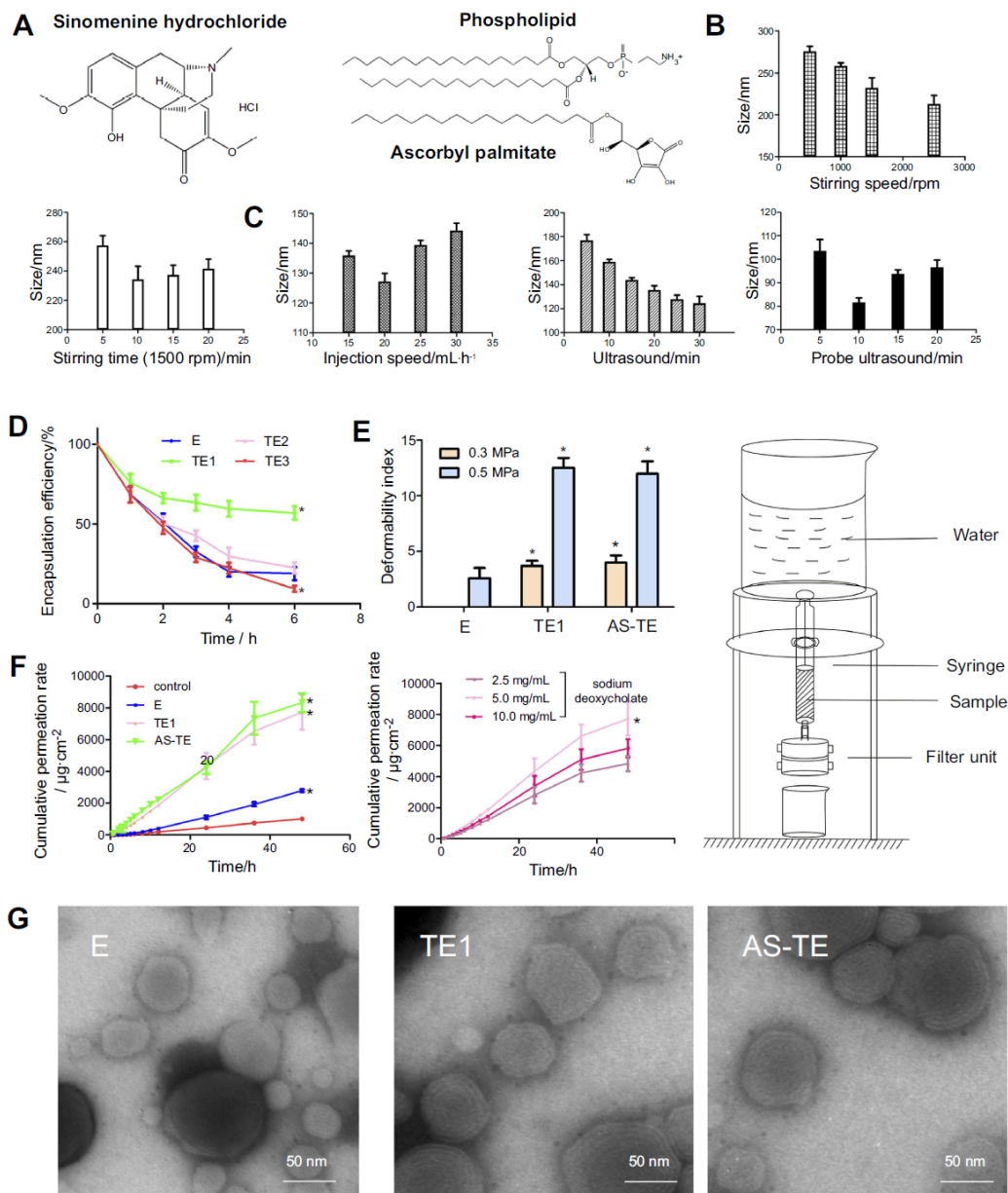
Figure 8. (A) The chemical structure of sinomenine hydrochloride, phospholipid, and ascorbyl palmitate. **(B)** The ethosomal sizes were prepared by different stirring velocities and time on the basis of the stirring-injection method. **(C)** The ethosomal sizes were prepared by different injection velocities, ultrasound time, and probe ultrasound time based on ultrasound-injection method. **(D)** The capsulation efficiency with different edge activators (*P<0.05 vs E). **(E)** The deformability indexes under 0.3 and 0.5 MPa were measured by self-made equipment on the right. (*P< 0.05 vs E). **(F)** The cumulative permeation rates in vitro of control (sinomenine hydrochloride normal saline solution), E, and TE1, containing different concentrations of sodium deoxycholate, and AS-TE. (*P <0.05 vs control and TE1 containing 5.0 mg/mL of sodium deoxycholate). **(G)** The TEM images of E, TE1, and AS-TE. **Abbreviations:** E, ethosome; TE, transethosomes (ethosome containing sodium deoxycholate (TE1), tween-80 (TE2) and oleic acid (TE3)); AS-TE, antioxidant surface transethosome. (Adapted from [146]).

1.3.3 Flavosomes

Flavosomes, transferosomes containing also the flavonoids quercetin and dihydroquercetin have been described for the delivery of non-steroidal anti-inflammatory drugs like meloxicam, with an enhanced skin permeability, reducing the side effects and by co-enhancing drug efficacy as they are also natural anti-inflammatories[149].

1.3.4 Glycerosomes

Glycerosomes, are alternative carriers for transdermal delivery, containing phospholipids, water and high concentration of glycerol. Diclofenac sodium salt was introduced in glycerosomes showing an enhanced skin penetration. Glycerol addition up to 20-30% acts as an edge activator with glycerosomes, being more fluid than liposomes, improving drug's efficacy and without causing skin toxicity[150]. Modified glycerosomes containing essential oils seem very promising due to essential oil's penetration effect and hypotoxicity. *Speranskia tuberculate* essential oil was used for the transdermal delivery of the hydrophilic drug paeoniflorin, improving the drug accumulation in the synovium. Specifically, it was found 3 times more drug accumulation in the synovium compared to conventional glycerosomes. Also, the transdermal flux as it is shown in Figure 9., was found 1.4, 1.6 and 1.7 times more than glycerosomes, liposomes and tinctures responsively[151].
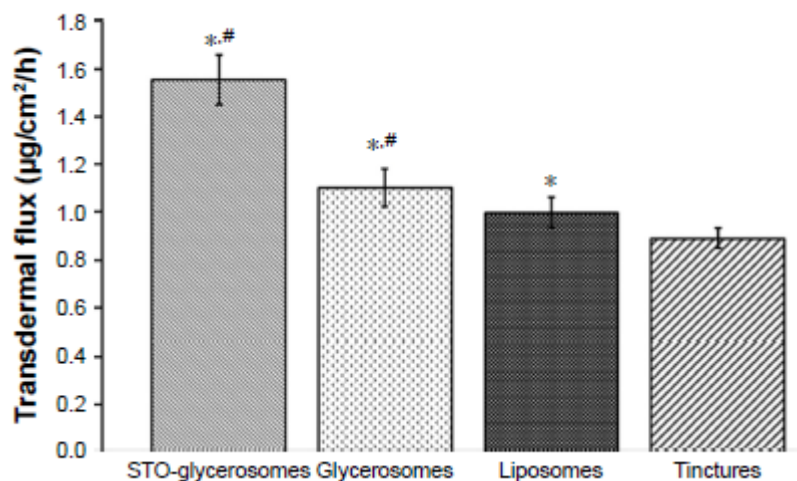
Figure 9. In vitro transdermal fluxes of paeoniflorin from different preparations (n=6; STO, *Speranskia tuberculata* essential oil) (*P,0.05, compared with tinctures; #P,0.05, compared with liposomes). (Adapted from [151]).

1.4 Lipid Nanoparticles

Lipid nanoparticles (LNPs) are composed of a dispersed lipid phase and a surfactant. They are synthesized from biocompatible triglycerides in their solid lipid core that offer an enhanced protection of API and a low surfactant concentration for a better stability[152–154]. In terms of thermodynamic stability, the length of triglyceride and the type of the added surfactant affect the crystallinity of the carriers. For example, polymorphic transitions are slower in longer chains[155]. Solid lipids can destabilize and expulse the drug from solid lipid nanoparticles (SLNs) due to the polymorphic transitions and the crystallization of lipid matrix that take place. They are not considered as the best option for controlled release of APIs because they melt in body temperature[156]. Also, the solubility of drugs in solid lipids is low and as a consequence limited drug loading and EE% are observed[157]. The second generation though, nanostructured lipid carriers (NLCs), overcomes these limitations through the combination of both solid and liquid lipids. The introduction of liquid lipids in the solid matrix generally creates imperfections in the crystal lattice of nanoparticles and improves the drug loading, stability, solubility and offers the possibility for modulation of their release properties[152,153,158,159]. Generally, SLNs and NLCs are loaded in hydrogels for their effective transportation through the skin[160]. SLNs and NLCs

combine the properties of emulsions, liposomes and polymeric nanoparticles. The advantages of using this type of carriers is mostly their ability to be used in damaged skin as the lipids that are composed of, are non-irritative, generally recognized as safe (GRAS) or accepted by the regulatory authorities[153].

Enhanced skin absorption of SLNs is given to their ability to form a lipid layer that adheres to the skin surface. The skin penetration of SLNs depends on their size but also on their crystallinity. Higher crystallinity improves the formation of the lipid film and thus the skin penetration[161]. Several studies have been performed for the transdermal delivery of APIs with SLNs. For example, SLNs composed of palmitic acid were prepared for the transdermal delivery of ivermectin and an improved stability, high EE and sustained release were reported. Also, improved skin penetration was attributed to the amorphous phase of the carriers[162]. Quercetin SLNs composed of palmitic acid and 2% Tween-80 were also studied. The optimal formulation was selected according to the highest occlusive effect, that was attributed to its small size. Also, it presented 5 times higher skin penetration than quercetin dissolved in PEG. Generally, it was observed that occlusive properties increased by a decrease in the particle size due to the increase of the surfactant content and the lowering of interfacial tension between lipid and aqueous compartments[163]. Additionally, a SLN-based transdermal patch was designed for the transdermal transportation of colchicine for the treatment of gout with an improvement of bioavailability near 3 times more than the colchicine patch without SLNs[164]. Furthermore, SLNs in the form of o/w microemulsions were formulated for the transdermal delivery of aconitine. The enhancement of the transdermal flux as well as the improved anti-inflammatory and analgesic effects compared to the ethanol tincture were proved in vitro and in vivo[165]. In another report, SLNs incorporated in a hydrogel film composed of hydroxypropyl methylcellulose (HPMC) and chitosan for the transdermal delivery of avanafil, improving its poor solubility and bioavailability[166]. Finally, curcumin was introduced in SLNs in a thermoresponsive hydrogel made from Pluronics. The added value of this type of hydrogel was the transformation from sol to gel state at 29.3°C, near skin temperature. Also, xanthan gum was used offering skin adhesive properties. In this

system, curcumin exhibited enhanced penetration compared to curcumin ethanol solution[167].

Furthermore, several reports highlight the superiority of NLCs as platforms for the transdermal delivery of drugs. NLCs can be categorized in 3 types based on their lipid composition. The first and main category is a mixture of both solid and liquid lipids enhancing the drug loading through the imperfections they create in the crystal lattice. The second category of amorphous NLCs includes lipid mixtures that are converted to solid matrix at room temperature possessing poor recrystallization ability and thus avoiding the risk of drug expulsion. The last category consists of different liquid lipid compartments inside a solid lipid matrix offering high loading capacity, improved drug solubility in oily areas and enhanced sustained drug release[157]. Olanzapine and simvastatin were encapsulated in NLCs inside a transdermal patch with a considerable reduction of drug's toxicity. Several PEs were combined with NLCs and the best results in terms of permeation rate were found with PEG[168]. Bupivacaine was introduced in linoleic acid-based NLCs modified by HA and PEG for local anesthetic application. These carriers exhibited 2.5 times higher transdermal penetration than free bupivacaine, prolonged the anesthetic effect and lacked cytotoxicity[110]. NLCs were also used for the delivery of anti-alopecia compounds (minoxidil and finasteride) with synergistic effects and good physical and chemical stability. For finasteride, higher loading efficiency was found compared to minoxidil. For minoxidil, a more sustained effect was observed, as finasteride is very lipophilic and after 24h it cannot be released from nanocarriers. However, although these systems were tested for their transdermal application, due to the low skin penetration that finally was observed, they are suggested for dermal applications[169]. Pioglitazone was introduced in NLCs increasing the skin penetration about 3 times more and improving the release profile and the bioavailability by 2 times more than the oral dosage form. For the highest solubility of drug, solid and liquid lipids, apifil and labrasol were respectively selected, along with Tween-80 surfactant[170]. NLCs composed of stearic and oleic acid as solid and liquid lipids respectively, as well as lecithin and taurodeoxycholate as surfactant and co-surfactant, were used for the delivery of donepezil through the skin with improved permeation rate[171]. NLCs-based

gel was studied for the transdermal transportation of diclofenac sodium and an improved drug penetration ability was observed. Small particle size achieved a better controlled release, skin permeation and final efficacy[172]. Moreover, NLCs were studied for the transdermal delivery of tenoxicam, a non-steroidal anti-inflammatory drug, promising for replacing oral formulations, by achieving a site-specific targeting with no systemic side-effects. NLCs were also entrapped in a hydrogel for achieving a longer stay in the administration site, without agglomeration[173]. Fan et al. studied the encapsulation of docetaxel in NLCs. As docetaxel shows very low solubility, nicotinamide was also used for the co-delivery. Thus, the complex docetaxel-nicotinamide encapsulated in NLCs possessed a better skin permeation of the encapsulated docetaxel alone[174]. Methotrexate incorporated in NLCs and in the presence of chemical enhancer, a-terpineol, was proposed for the treatment of rheumatoid arthritis, reducing inflammation and enhancing the therapeutic effects[175]. Celastrol and indomethacin were co-incorporated in NLCs exhibiting anti-arthritic activity and anti-inflammatory effect without adverse-effects. In this case, as a liquid and a solid lipid Labrasol and Precirol ATO 5 were used respectively, and Cremophor RH40 was selected as surfactant[176]. Lastly, the acid-labile drug lansoprazole was introduced in NLCs. SA ensured the drug stability in NLCs and SDS was used for disturbing lipid organization in the SC and thus facilitating the skin penetration. Overall, the combination of anionic SDS and cationic SA inhibited NLCs aggregation and offered a sustained release of the drug. PEs isopropyl myristate and menthol were also used[158].

Many studies have been done comparing the effectiveness of NLCs and SLNs. Bhaskar et al. compared SLNs and NLCs in polymeric hydrogels and in dispersions for the transdermal delivery of flurbiprofen. The bioavailability of both SLNs and NLCs in gel was near 4.5 times more than the conventional oral dosage form and the half-life was increased significantly. Also, a better prolonged release was achieved with the hydrogel formulations[153]. Capsaicin was entrapped in NLCs and SLNs with an excellent colloidal stability following a zero-order kinetics and thus a sustained drug release, more rapid transdermal penetration and reduction of skin irritancy[177]. Gu et al. studied SLNs and NLCs for the transdermal delivery of triptolide. They observed a

higher loading capacity for NLCs due to the less-ordered structure resulted from the combination of both liquid and solid lipids. As a result, in NLCs the drug penetration rate was higher and a stronger interaction with the skin was found. Triptolide LNPs were more effective than triptolide solutions because they could be endocytosed in comparison with the solution which could be absorbed only by passive diffusion[162]. Finally, no results of skin irritancy were observed[178]. A thorough study of formulation characteristics like the drug and oil concentration and the different types of oils in NLCs and SLNs for the transdermal delivery of all-trans retinoic acid was performed. ATRA as it is very lipophilic, it is more soluble in liquid lipids. Also, lower drug loading capacity was found for the LNPs composed of soybean oil due to ATRA solubility in the lipid area. The highest ATRA release rate was found for formulations with medium chain triglycerides and soybean oil. Higher drug permeation was found with SLNs than NLCs and suspensions. Smaller size of SLN resulted in higher flux of drug because of a more apparent occlusive effect. It was reported that oleic acids increased ATRA permeation in both SLNs and NLCs[179]. Between SLNs based on Precirol®, NLCs and nanoemulsions, the first were selected as the best formulation for the transdermal delivery of olanzapine with a small size, a good stability and a narrow size distribution. Generally, regarding the ratio solid to liquid lipid, as it was decreased, the average particle size was also decreased, more negative values of zeta potential were found and the occlusive effect was reduced. Also, with higher liquid lipid ratio, the drug release is faster, although this depends on a lot of parameters. For example, SLN due to their bigger particle size may decrease the drug diffusion from their surface. Also, in oleic acid-LNPs, although having a greater ratio of liquid lipid, the drug release is lower due to the enhanced solubility in oleic acid; in this case, solubility matters more than size. SLNs showed the best occlusive effect given to their greater crystallinity which resulted in a faster penetration rate compared to oleic acid LNPs which due to the high solubility of drug in oleic acid, they were expected to have the fastest skin penetration effect[180]. The study of solid lipid ratio on both SLNs and NLCs revealed the effect on the properties and the superiority of NLCs in terms of stability and transdermal penetration of flurbiprofen with the same amount and type of used surfactants. Also, in both types the same solid lipid was used (Compritol® ATO 888). The increase of liquid to solid lipid ratio improves the EE and the stability of the drug.

Also, it was found that SLNs have a decreased occlusion factor because of the bigger particle size in contrast to small-sized NLCs with stable occlusion factor at the initial value. Skin penetration was highly dependent on the particle size of carriers which is correlated to the liquid to solid lipid ratio. Also, during storage of 3-months, it was found that skin penetration of flurbiprofen from SLNs was decreased in contrast to NLCs which were found more stable[181].

1.5 Comparative studies

Among the reports of lipid-based formulations for transdermal delivery that have been presented above, the mechanism of action, the penetration enhancement and the final effectiveness differ a lot and depend on many parameters. For example, Chen et al. studied the effect of different lipid vesicles (conventional liposomes, invasomes and ethosomes) and of the application mode (finite and infinite dose) for the skin permeability for a model hydrophilic and lipophilic drug, carboxyfluorescein and temoporfin respectively. It was reported that when carboxyfluorescein was tested with both modes of application, ethosomes and invasomes showed better results in skin permeability, whereas for temoporfin, it was observed that it was deposited only in the upper skin layer for all carriers. Lipid vesicular systems were found to improve the penetration of hydrophilic rather than lipophilic drugs. Also, for hydrophilic drugs, carrier's composition plays a significant role regarding the drug deposition on the skin. Application mode affected indirectly the drug distribution through the degree of hydration of the SC[182]. Transferosomes and invasomes were also compared with conventional liposomes and it was found that invasomes possessed higher elasticity from transferosomes and both were more deformable than conventional liposomes. The drug permeation of calcein which was used as model of low molecular weight drug was enhanced a little by conventional liposomes whereas it was improved significantly by 2 and 7 times for transferosomes and invasomes respectively[183]. Another comparative study between conventional liposomes, ethosomes and transferosomes was performed for the transdermal delivery of ovalbumin and saponin, aiming at the design of a needle-free vaccination system. Ethosomes were

proved to be the best carriers, keeping a stable size and polydispersity index within a period of 2-months storage, as it is presented in Figure 10.[184].
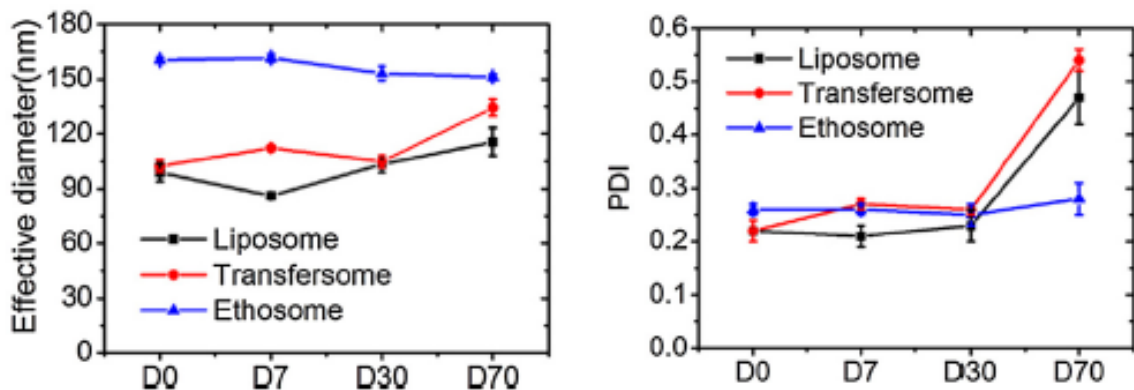

Figure 10. Size aging assay of lipid vesicles by DLS. (Adapted from [184]).

A comparison between liposomes, ethosomes and transferosomes was performed also for diclofenac sodium. It was found that transferosomes and ethosomes provided a better skin penetration, flux and permeability coefficient than liposomes, conventional gel and hydroethanolic solution[185]. In another report, ethosomes and transferosomes were compared in terms of size, surface charge, EE and drug release profile with conventional liposomes for the transdermal delivery of diflunisal. Improved anti-inflammatory response was found for the deformable vesicles compared to the conventional liposomes. More specifically, an ethosomal formulation was selected for further investigation due to highest EE and suitable size and surface charge[186]. Conventional liposomes, propylene-glycol liposomes and ethosomes were compared for the transportation of curcumin in the skin. Propylene-glycol liposomes exhibited the smallest particle size, lowest dispersion coefficient and highest encapsulation efficiency. Also, they showed the best anti-inflammatory effect, and thus were considered as the most promising carriers for the efficient transdermal delivery of curcumin[187]. Nanoemulsions (will be discussed in the next sections) with a fluid core, liposomes and SLNs with a solid core were studied for the transportation of retinyl palmitate. TEM images for all carriers transporting retinyl palmitate are presented in Figure 11. It was found that NEs possessed a higher flux than SLNs and liposomes, but liposomes exhibited higher skin retention than the other two

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categories. Also, NEs were found to cause a skin disruption. Due to drug expulsion in SLNs, liposomes and NEs improved by far the skin permeation in deeper layers[188]. Triamcinolone acetonide was introduced in biocompatible LNPs composed of poly (lactic-co-glycolic acid) and caused an apoptosis to human skin fibroblasts. Compared to common liposomes they showed an increased EE. Also, their skin permeability was found 2 and 40 times more than conventional liposomes and commercially available suspensions respectively[189].
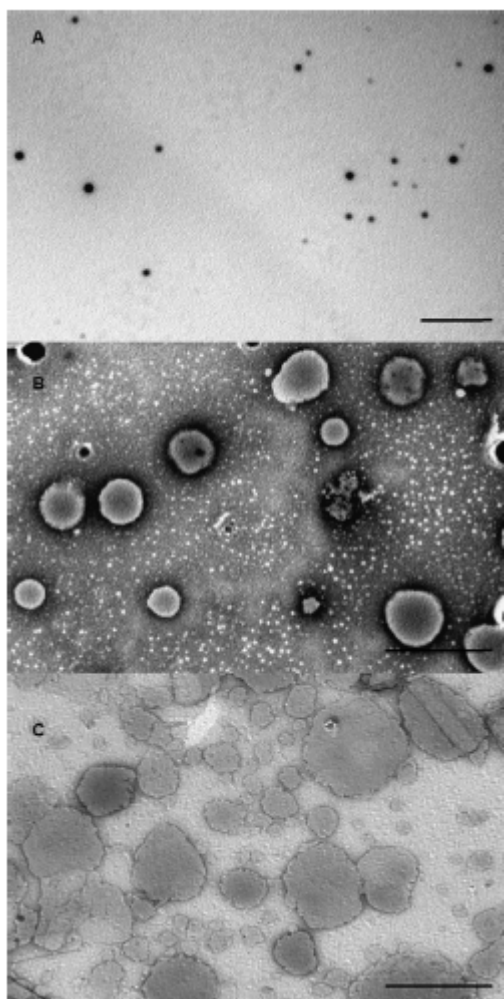

Figure 11. Transmission electron micrographs of RP in nanoemulsions, bar length 100 nm **(A),** SLNs, bar length 500 nm **(B),** and LPs, bar length 500 nm **(C). Abbreviations:** RP, retinyl palmitate, SLNs, solid lipid nanoparticles, LPs, liposomes (Adapted from [188]).

2. POLYMER-BASED NANOSYSTEMS

Polymer-based nanosystems, mainly micelles, polymersomes, dendrimers, nanoparticles and nanogels hold a great promise for transdermal applications due to their outstanding properties. The above systems can protect effectively the drug from degradation, increase the skin penetration and absorption and control the overall pharmacokinetic profile. Also, due to their multiple options for functionalization, their selective accumulation in tissues can be controlled. Compared to lipid-based nanosystems, they show a better long-term stability during storage conditions and they are reported promising to solve several formulation problems. Another remarkable property, is their stimuli responsiveness (pH, T, redox) in some cases, which can be advantageous in smart transdermal drug delivery systems. Finally, the mechanism of drug penetration of polymer-based nanosystems is still under investigation. However, the follicular pathway seems to be the main pathway through which these systems can act as local drug reservoirs[190–192].

2.1 Polymeric micelles

Polymeric micelles are composed of natural, synthetic or semi-synthetic amphiphilic block copolymers (diblock, triblock or grafted polymers) that self-assemble and are formed above the critical micelle concentration (CMC). They possess a hydrophobic core that acts as a depot of hydrophobic compounds and a hydrophilic shell that enhances the colloidal stability of the system. They exhibit a small size around 10-200nm and a spherical shape. They can increase the stability and solubility of the API exhibiting at the same time low toxicity and enhanced biocompatibility. The most important advantage for them is their enhanced skin penetration ability, given to their small size[193,194].

Zhou et al. studied the loading of curcumin in methoxy-PEG-poly (ε-caprolactone) micelles and by mixing them with a-cyclodextrin (CD) solution they managed to enhance the water solubility and poor skin penetration ability. The resulted

supramolecular hydrogel can be used for the treatment of dermatitis achieving enhanced skin deposition, improved efficacy, and better stability, displaying also erosion ability in aqueous environment[195].

Moreover, polymeric micelles Soluplus®, composed of grafted ampipathic copolymers, polyvinyl caprolactam (57%) and polyvinyl acetate (30%) as well as PEG (13%)[196], were used as adjuvants for tetanus toxoid transcutaneous immunization. These micelles induced an improved Ab production due to the enhanced uptake of antigen by the skin, maybe by Langerhans cells[197]. Encapsulation of epigallocatechin gallate in polymeric micelles based on Poloxamers, offers enhanced stability and skin penetration for a better final efficacy. Polymeric micelles were introduced in oil-in-water (o/w) emulsions for a better control of the release and it was found that glycerides and esters provided the best results. However, the drug stability in this type of carrier systems needs to be confirmed[198]. L-DOPA was encapsulated in micelles composed of labrasol, glyceryl oleate as surfactant and propylene carbonate as co-surfactant for the improvement of drug's low skin permeability and poor solubility and the increase of transdermal permeation and systemic distribution. This self-assembling nano-micellar system is promising as it can transport a high cargo of L-DOPA, for a constant dopaminergic stimulation, limiting motor fluctuations. However, its exact mechanism needs yet to be investigated[199]. A mixed micellar system was used for the transportation of indirubin in order to improve its poor solubility and low bioavailability and thus to increase the efficacy and reduce the required doses. The above system was composed of surfactants Kolliphor® EL for a better bioavailability via drug adsorption on the skin and Tween 80 for the increase of skin permeation ability. As co-surfactant, (2-hydroxypropyl)-β-CD was used as a solubility enhancer. Overall, the micellar system is very promising for the transdermal delivery of poorly soluble drugs with a small-sized droplet and is safe as all the excipients are FDA-approved[200]. PEO-PPO-PEO polymeric micelles improved the bioavailability and gene expression as well as increased the circulation time of plasmid containing the gene for *β*-galactosidase through its transdermal administration. More specifically, the hydrophilic character of poly (ethylene oxide) (PEO) and the hydrophobic nature of poly (phenylene oxide) (PPO) facilitate the self-assembly in polymeric micelles with

enhanced stability and prolonged release profile of the gene. Therefore, the increase of gene absorption and expression in several tissues was achieved[201].

2.2 Dendrimers

Dendrimers are multivalent branched polymeric structures composed of synthetic polymers that are evolved radially from a central core. The number of branching points from the central core gives the generation (G) of a dendrimer. Their synthesis is characterized by the advantage of controlling the final molecular weight and chemical composition and thus the biocompatibility and pharmacokinetic profile of these carriers. At the same time, their synthesis may be difficult as there are multiple steps and the cost may be increased. However, these structures are structurally and chemically homogeneous, possessing high ligand density and various options for modification. Finally, they can be degraded in a controlled manner[202,203]. Yang et al. studied the effect of different physicochemical properties as size, hydrophobicity and surface charge on the skin penetration of Poly(amidoamine) (PAMAM) dendrimers. They found that smaller PAMAM dendrimers (G2) exhibit better results than larger ones (G4). Also, surface modification plays a significant role, as acetylated or carboxylated dendrimers show improved skin permeability and follow an extracellular pathway and a rapid diffusion due to their charge repulsion with the cell membrane. This phenomenon renders them beneficial for systemic administration applications, where deep and fast penetration of APIs is needed. On the other hand, amine-terminated dendrimers that exhibit strong skin retention and internalization to dermal and epidermal skin layers, as it can be seen in Figure 12., are suitable for localized transdermal applications. Lastly, conjugated dendrimers with oleic acid were studied, and it was found that they increase the skin absorption and retention due to the increase of their hydrophobicity. According to the authors, the optimal dendrimers are those with log P values between 1 and 3[204].
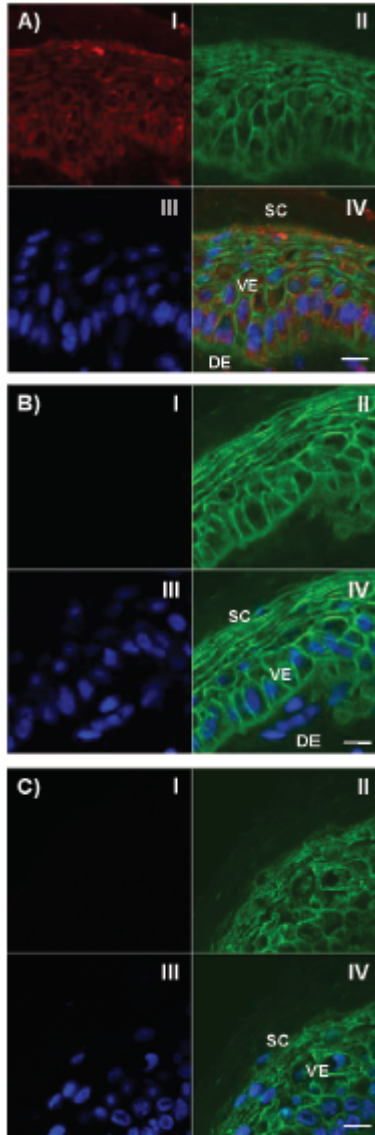
Figure 12. Confocal images of cross sections of microtomed porcine skin layers after 1 h treatment with: **(A)** G2-RITC-NH2, **(B)** G2-RITC-COOH, or **(C)** G2-RITC-Ac. The dendrimer conjugates (I,red); cell membranes stained by WGA-AF488 (II, green); nuclei stained by DAPI (III, blue); and merged images of all three channels (IV) are shown in each quadrant. Note that G2-RITC-NH2 strongly interacts with the epidermal/dermal cells whereas G2-RITC-COOH and G2-RITC-Ac do not. Scale bar: 10 µm. **Abbreviations:** SC, stratum corneum; VE, viable epidermis; DE, dermal layer. (Adapted from [204]).

G5-PAMAM dendrimers conjugated with TAT-peptide were used for the transdermal delivery of a DNA plasmid which encodes avian influenza virus H5 protein, holding a great promise for transdermal gene delivery. PAMAM-dendrimers were selected due to their intrinsic ability of transfection into cells and their high-organization architecture. TAT-peptide increased the penetration ability of the plasmid and thus its easier uptake from cells. As these polyplexes carry positive charge, due to the cationic

peptide TAT, they can interact with the negatively-charged cell membrane and improve the cellular uptake. Also, this type of system can be easily produced and with low cytotoxicity, which renders it promising as a non-viral vector for transdermal application[205]. G3 and G4 PAMAM dendrimers were investigated as transdermal carriers for 8-methoxypsoralen and a deeper skin penetration as well as an increased dermal and epidermal concentration of the drug were found. Also, G4 PAMAM achieved better results than G3 PAMAM dendrimers, improving the efficacy of photochemotherapy[206]. PAMAM dendrimers of generations G2, G2.5, G3, G3.5 and G4 were tested as solubility enhancers for vitamin B2. G2-PAMAM dendrimers were found to be the best option for enhancing the skin permeation of vitamin B2 due to the enhanced hydrophilicity and small size. G2 and G3 dendrimers could be used in hydrogels and emulsions to control the release of Vitamin B2[207]. PAMAM dendrimer conjugated with TAT peptide was also tested as a transdermal carrier of a plasmid, expressing the pBud-H5-green fluorescent protein. In parallel, interferon regulatory factor 3 (IRF3) was used as genetic adjuvant and the overall potential of PAMAM dendrimers in gene delivery as vaccine systems was investigated. The findings reported a better skin permeation, an improved transfection efficiency and a low cytotoxicity that render PAMAM dendrimers quite promising in their application as DNA vaccine delivery systems[208]. The efficacy of this DNA vaccine delivery system via transdermal administration is presented in Figure 13. Janus type G1 and G2 dendrimers composed of poly (propyl ether imine) and oleic acid through ester and amide bonds were investigated for skin penetration enhancement of the model hydrophilic drug diclofenac sodium. These oleodendrimers didn't show any toxicity effect. Key parameters that were considered, were the generation of dendrimer, the type of linkage between oleic acid and dendrons, and the lipophilicity of the system. More specifically, higher generation, increased lipophilicity, and the ester linkage between oleic acid and dendrons proved to exhibit the best results in terms of skin permeation[209].
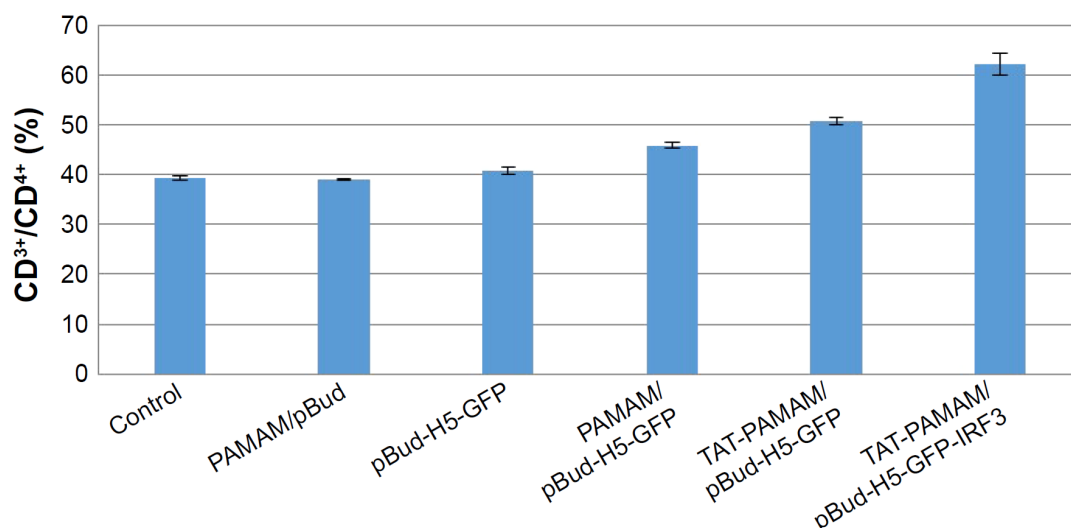
Figure 13. Population of CD3+/CD4+ T cells in inoculated mice with PAMAM/pBud, pBud-H5-GFP, PAMAM/pBud-H5-GFP, TAT-PAMAM/pBud-H5-GFP, and TAT-PAMAM/pBud-H5-GFP-IRF3. **Notes:** Vaccination with H5-GFP plasmid DNA using TAT-conjugated PAMAM dendrimer showed the significant population of CD4+ T cells compared with native PAMAM dendrimer. The population of CD4 in vaccination with H5-GFP-IRF3 plasmid DNA using TAT-conjugated PAMAM dendrimer was significant compared to the other resultant groups. **Abbreviations:** GFP, green fluorescent protein; IRF, interferon-regulatory factor; PAMAM, polyamidoamine; TAT, transactivator of transcription. (Adapted from [208]).

Peptide dendrimers are composed of amino-acids linked with amide bonds and possess a radial-branched structure of a peptidyl core and surface functional groups[210]. They are less toxic, as they are biodegraded from peptidases into single amino acids instead of toxic acrylates as in the case of PAMAM-dendrimers[211,212]. Asenapine maleate was introduced in arginine-terminated peptide-dendrimers that were conjugated with liposomes. A higher transdermal penetration was found for the lipidated dendrimers compared to liposomes without dendrimer conjugation because of the cationic charge of dendrimers and the fluidizing effect they may exhibit. Higher bioavailability and longer retention of the API with low cytotoxicity was reported for this type of carriers compared to the oral dosage formulation. The addition of liposomes to dendrimers leads to a better transdermal delivery, given to their intrinsic ability to interact with the cell membranes resulting in easier internalization. Comparing two formulations with 4^+ and 8^+ charge, the one with the higher charge

showed better results in terms of skin penetration due to the higher surface charge that possesses. Also, except for the higher charge, the lipidation with oleic acid for the second formulation, contributed to this phenomenon, as oleic acid acts as a PE. Both formulations were stable for 4 months in terms of size, polydispersity index and drug content[213]. In another report, arginine and histidine terminated peptide-dendrimers were investigated for the skin penetration through iontophoresis depending on their weight. As they are charged, they can be transported successfully through the skin with the application of electric current increasing the flux significantly[211]. Additionally, peptide dendrimers were tested for the transdermal delivery of antioxidants silibinin and epigallocatechin-3-gallate. More specifically, the 8+ peptide dendrimer composed of glycine, proline and lysine exhibited an improvement in the skin permeation and retention of both antioxidants[214].

2.3 Polymeric Nanoparticles

Polymeric nanoparticles were studied in several reports as promising transdermal carriers due to their unique properties as well as most often due to their biodegradability and biocompatibility (chitosan, PLGA, PLA etc.). More specifically, chitosan nanoparticles which are characterized by increased stability, biocompatibility and mucoadhesive properties[215] will be described firstly. Propanolol-HCl was introduced in chitosan nanoparticles along with tripolyphosphate as a cross-linker and then dispersed in gel composed of Poloxamer 407 and Carbopol 940. The optimal drug delivery system with size around 190 nm and zeta potential +35 mV exhibited sustained release properties and improved the systemic bioavailability of the drug[216]. Imiquimod was entrapped in chitosan nanocapsules, in the presence of a novel excipient that acted as a PE, Compritol 888ATO®. Raman microscopy was performed for the evaluation of the ability of chitosan nanocapsules to disrupt the SC and of the kinetics they follow. A time period of around 50 min was needed for nanocapsules to permeate the skin and 24 hours after, the drug was found in the deeper skin layers. This system was also stable in terms of size and polydispersity during 48h and for 2 months after lyophilization[217]. Moreover, baicalein introduced in lecithin-chitosan

nanoparticles which resulted in sustained release, prolonged skin retention, enhanced skin penetration and good stability without any skin irritation. This mixed system was formed by a self-assembling process via electrostatic interactions between the negatively-charged lecithin and the positively-charged chitosan. The combination of these two parts is beneficial as the inner core of lecithin is lipophilic for the entrapment and solubilization of lipophilic drugs and the chitosan shell, on the other hand, is hydrophilic and promising for improving the penetration of SC barrier. Thus, the mixed system (Figure 14.) can combine the properties of both components enhancing the retention of drug in the skin via a more sustained release[218]. Khalil et al. complexed hydrophobic warfarin in β-CD complexes in order to be possible then to incorporate the whole complex in hydrophilic chitosan nanoparticles. They managed to entrap the drug with a very high EE of 94% and reported a sustained release profile and an improved skin permeation ability. Overall, the described carrier holds a great promise for transdermal drug delivery of lipophilic drugs as the role of chitosan is to increase the penetration ability and to control the release rate of the API, whereas β-CD is used as a solubility enhancer for an easier drug incorporation[219]. Lastly, Nair et al. evaluated the effectiveness of chitosan nanoparticles with intrinsic skin permeation ability, loaded with curcumin and reported an enhanced drug release, transdermal permeation and cell viability compared to curcumin solution[220].
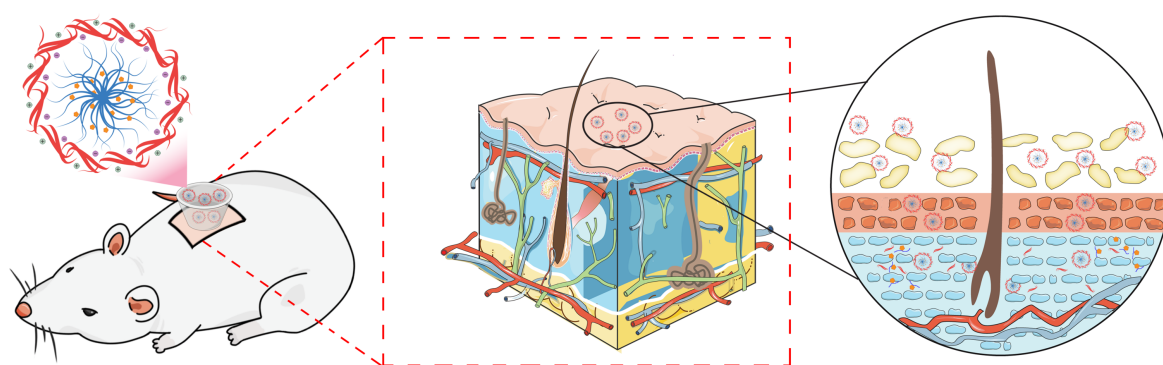

Figure 14. Schematic overview of BPC-LCNs that improves the retention of baicalein in the skin and further penetrates the barrier of stratum corneum. **Abbreviation:** BPC-LCNs, lecithin/chitosan nanoparticles loaded with baicalein-phospholipid complex. (Adapted from [218]).

Furthermore, biocompatible and biodegradable alginate nanoparticles possess an increased mechanical strength and coated with chitosan show increased hydrophilicity[221]. Pirfenidone was encapsulated in chitosan-sodium alginate nanogel composed of natural polymers with a significant increase of its skin permeation ability[222]. Alginate core nanoparticles coated with chitosan were also used for the transdermal delivery of rabeprazole through a water-in-oil (w/o) nanoemulsification method and the formulation of transdermal patches that showed an enhanced skin permeation and a sustained release of the API[223].

Synthetic poly (lactic-co-glycolic acid) (PLGA) nanoparticles derive from the co-polymerization of lactic and glycolic acid and they are characterized by biodegradability, biocompatibility and prolonged release properties, being promising for the transdermal delivery of very hydrophilic APIs[224]. HA-grafted PLGA nanoparticles were used to encapsulate minoxidil for alopecia treatment and they were found to significantly increase the drug loading capacity without any dose-dependent cytotoxicity[225]. Also, 50 and 100 nm PLGA nanoparticles were used for the transdermal delivery of hydrophobic indomethacin and a high accumulation of the drug in the SC was found. 50nm nanocarriers offered a deeper skin penetration and a higher drug concentration almost 1.7 times more than the 100nm nanovehicles[226].

Other types of polymeric carriers had also been investigated. For example, hydrophobic poly (caprolactone) (PCL) polymeric nanofibers were studied for the transdermal delivery of the hydrophilic cyanocobalamin. Its prolonged release was achieved by nanofibrous reservoir as well as the protection of the vitamin due to the hydrophobic nature of the polymer which improves the durability[227]. Chitosan, poly (lactic acid) (PLA) and PCL were also used as biodegradable polymers for the formation of transdermal patches to deliver repaglinide, resulting in 75 times higher bioavailability than the oral administration. They hold a great promise for diabetic patients, since they prolong the drug release, reducing the dose frequency and the cost at the same time[228]. Polymeric nanocarriers synthesized from amphiphilic co-polymers PEO and PCL, mannosylerythritol lipid and TAT-peptide were proposed for enhanced transdermal delivery and cellular uptake. More specifically,

mannosylerythritol lipid (MEL) interacts with the methoxy group of PEO-b-PCL through dipole-dipole interactions. Its asymmetric molecular geometry provided the polymeric vehicle with flexible characteristics, forming a gel-like dispersion. Also, the TAT peptide linked to MEL improved the cellular uptake due to micropinocytosis and caveolae-endocytosis[229]. In another report, PCL was used as the polymeric core modified with stearic acid as a FA for the transdermal delivery of betamethasone and oleic acid as a coating. The above hybrid nanoparticles offered prolonged release of the drug, improved protection from destruction and enhanced effectiveness. Also, improved skin permeation without any irritancy was reported[230]. Lastly, polymeric nanosheets with high biocompatibility have been proposed as novel topical or transdermal carriers for betamethasone valerate synthesized from PLA or PLGA. These formulations possess a high adhesiveness and enhanced moisture permeability, being thus ideal for long-term application in skin. The selected polymers as well as the drug concentration and the presence of a controlled-release membrane, controlled the release of drug[231].

2.4 Polymersomes

Polymersomes are similar to the vesicular structure of liposomes containing a hydrophilic interior, but they are composed of amphiphilic block copolymers and they possess a higher mechanical strength due to the higher molecular weight of polymer chains. Also, by controlling their molecular weight is possible to control their mechanical properties and permeability[232]. They seem to be promising in transdermal drug delivery due to their enhanced mechanical strength which results in an excellent drug protection and sustained released. The presence of entangled polymeric chains correlates with a very high energy needed for vesicle rupture. It was reported that polymersomes can permeate from pores that are 8 times smaller than their size in contrast to liposomes that can be squeezed up to half their size and transferosomes that reduce their size up to a quarter[15,95,233]. Pegoraro et al. investigated polymersomes for their transportation across small pores, with promising application for their use in transdermal drug delivery of large macromolecules such are dextran

and antibodies. These flexible vesicles were reported to be sensitive to external gradients such are capillarity, osmotic gradient or hydration. More specifically, $PMPC_{25}$-$PDPA_{200}$, $PMPC_{25}$-$PDPA_{70}$ and PEO_{113}-$PDPA_{70}$ were investigated. Comparing the different external polymer brush PMPC and PEO, PEO_{113}-$PDPA_{70}$ were found to end fragmented during their translocation due to a more hydrophobic nature. Also, comparing $PMPC_{25}$-$PDPA_{200}$ and $PMPC_{25}$-$PDPA_{70}$, the first showed a slower penetration due to a more rigid structure. Thus, $PMPC_{25}$-$PDPA_{70}$ was found the most suitable system for the intact transportation of large macromolecules across 50nm pores. Remarkably, the translocation efficiency was higher in 400 nm-sized $PMPC_{25}$-$PDPA_{70}$ compared to 200 and 100 nm due to the higher flexibility that is connected with larger structures[234].

3. NANOEMULSIONS

Nanoemulsions (NEs) are considered the heterogeneous systems where one liquid is dispersed inside another in the form of nanosized droplets below 500nm. The oil-water interface of these systems is stabilized by surfactants and co-surfactants. Actually, the reduce of interfacial tension is given to their amphiphilic chemical nature as they possess a hydrophilic head and a hydrophobic tail[235,236]. Regarding the type of surfactant, the size and rheological properties of the NE differ[237]. The three main categories of NEs are the o/w (oil phase dispersed in continuous aqueous phase), w/o (water droplets dispersed in continuous oil phase) and bi-continuous (microdomains of both oil and water continuous phases)[238]. NEs are very promising for enhancing the bioavailability and solubilization of poorly soluble drugs, especially, o/w emulsions when applied to skin, as they improve the absorption of lipophilic drugs[235].

Generally, they are characterized by a good colloidal stability due to the small droplet size that prevents coalescence and flocculation and lowers the surface tension. Also, their small size and high surface energy enables their easy skin penetration. They are manufactured easily with low energy input and they possess a long shelf life. However, compared to other nanoformulations there are less NEs approved or under clinical

trials[236,239,240]. In our opinion, the main reason for their limited clinical translation is the lack of deep understanding of the interfacial phenomena that are present in these systems, which should be clarified and quantified for the approval process of the medicine[239]. Interfacial energy plays a key role in NEs in terms of their stability in the shelf and in the human body and effectiveness.

The size, the charge and the nature of the dispersion phase of the final NE-based formulations can be controlled regarding the intended drug delivery application. For example, the transdermal delivery is reported for NE-droplets less than 50nm[241]. A comparison of NE size has shown that size plays a key role on the transportation across the skin. More specifically, a size of 80nm is correlated with a skin diffusion but not with the penetration of the epidermis. 200nm NEs exhibit a moderate penetration ability and are transported via the hair follicular route. Bigger NEs of 500nm do not penetrate the SC and they are transported only through the hair follicle channels[242].

Also, in another report the effect of different concentrations of Carbopol 934, a polymeric thickener, which transforms NEs into NE-gels was studied. The formulations contained terbinafine and citral as model drugs and their stability, irritation, skin penetration profile and drug delivery pathways were investigated. By controlling the concentration of Carbopol 934, it is possible to modulate the skin penetration regarding the therapeutic purpose and the necessity for either topical or transdermal formulations. It was reported that NE-gels, following an intercellular pathway, achieved a higher concentration of drugs in the SC compared to NEs alone. Also, they proved to be safer in terms of skin irritancy. The higher amount of Carbopol 934 was used in the formulation, the more the skin penetration ability was declined, the drug deposited mainly in the epidermis and dermis, the drug diffusion was altered from skin appendages to intercellular paths and a different histological response was observed[243]. Although the penetration pathway of these formulations is still unclear, in another report where ibuprofen NEs were studied for their penetration mechanism, it was suggested that they fuse with the SC in order to create new channels for the penetration of drugs in deeper layers[244].

Several reports described the use of o/w NEs for the transdermal delivery of several APIs. More specifically, the encapsulation of piroxicam in NEs composed of oleic acid, Tween 80 and ethanol can improve its low solubility and reduce the side effects associated with the oral delivery. Also, Carbopol 934 was used to transform NE to nanoemulgel with an increase of its viscosity. The above formulation is promising as it combines easy applicability and enhanced penetration ability with a high skin permeation rate, without the use of PEs and thus reducing the skin irritancy[245]. Similarly, sulconazole was incorporated in NEs composed of capryol 90 oil, labrasol surfactant and 1,2-propanediol cosurfactant and improved its intrinsic low water solubility, as well as enhanced the skin penetration resulting in better antifungal effects compared to commercially available antifungal agents as dimethyl sulfoxide (DMSO) solution containing sulconazole and miconazole cream[246]. NEs composed of caprylic acid, Tween-80, PEG and in the presence of Carbopol 940 for the formation of a gel were synthesized for the transdermal delivery of meloxicam with improved efficacy in anti-inflammatory therapy compared to the drug solution[247]. O/w optimized NE and NE-gel were developed for the transdermal delivery of bromocriptine mesylate, achieving a sustained drug release, high skin permeation, improved efficacy, safety and biocompatibility. Also, the bioavailability of the NE loaded in Carbopol gel was improved 274% compared to the NE alone[248]. HA oil-water-surfactant NEs with good stability were synthesized for the transdermal delivery of hydrophobic 10,11-Methylenedioxycamptothecin possessing small size, negative charge, flexible structure and controlled-release properties. The NEs contained HA-Glycerol-α-monostearate phosphate buffered saline (PBS) solution and methylene chloride as the continuous and dispersed phase respectively and Tween-80 and Span-20 as surfactants. The above NEs can be internalized by keloid fibroblasts and inhibit its proliferation without any toxicity to normal fibroblasts[249]. O/w NEs were also tested for the transdermal delivery of the anaesthetics imipramine and doxepin. Analgesic effects were found stronger for doxepin incorporated in NEs. NEs were composed of water, transcutol (solubilizer) and PEG as the water phase, labrasol and plurol oleique as non-ionic surfactant and co-surfactant respectively and isostearyl isostearate, oleic acid and D-limonene as the oil-phase. The above NEs were found stable for 3 months.

The best formulation was characterized by 45% aqueous phase, 40% surfactants and 15% oil phase[250]. Atorvastatin calcium with a very low bioavailability introduced in NEs synthesized from oleic acid, Tween 80 surfactant, PEG 400 co-surfactant as well as ethanol and limonene as PEs. The addition of PEs was necessary as NEs could not carry lipophilic drugs alone. The formulation exhibited a prolonged drug release and increased bioavailability. In vivo studies revealed that the follicular pathway was the main mechanism of NEs penetration[251]. Su et al. investigated through screening experiments the factors for the optimization of NEs for the delivery of ceramide IIIB across the skin. More specifically, the ratio octyldodecanol/ (Tween80: glycerol), the water content, the rate of addition and mixing and the temperature were tested. The optimal conditions for the lowest size and polydispersity index, according to Figure 15., were found to be a temperature around 41.49 °C, 1.74ml/min addition rate, 55,08 wt.% water content and 720rpm mixing rate[252].
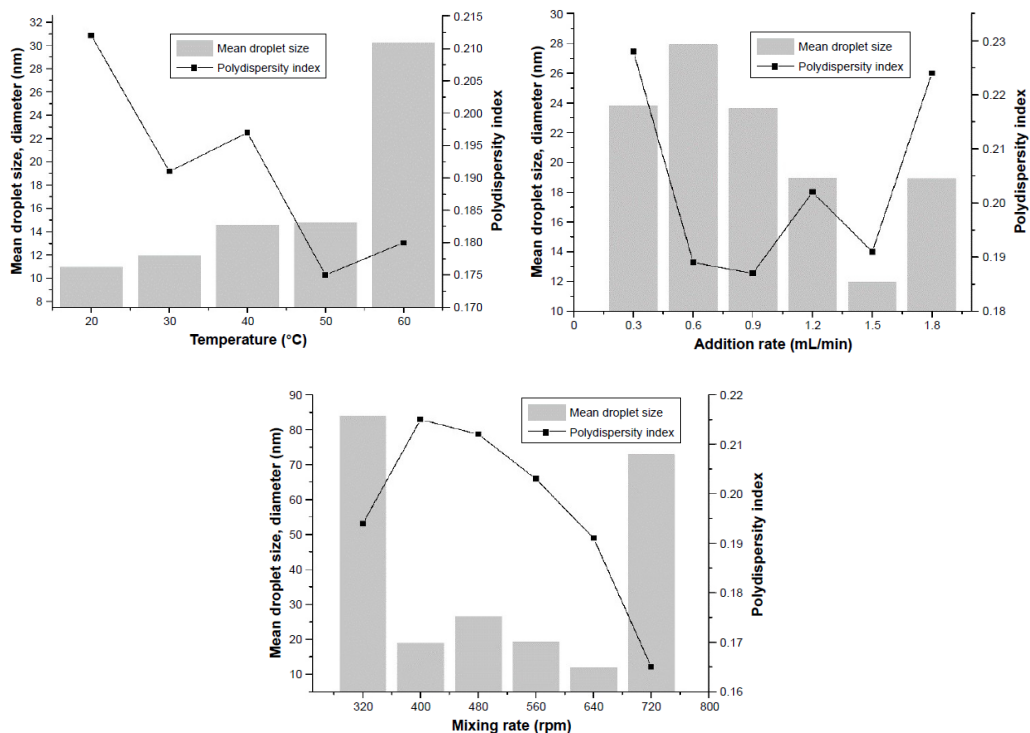

Figure 15. Effect of T, addition and mixing rate on mean droplet size and polydispersity index. (Adapted from [252]).

Although o/w NEs have gained more attention in the field of transdermal drug delivery, in another study w/o NEs were investigated for their effectiveness. Analytically, w/o NEs were synthesized for the transdermal delivery of the anti-inflammatory drug thiocolchicoside. The main pathway was the transfollicular route and it was finally observed an improvement of the biovailability and a reduce of side effects. Linseed and sefsol were used as the oil phase, and Span-80 and transcutol P as surfactant and co-surfactant respectively. The optimized formulation showed a 5 times higher transdermal flux of the drug compared to the control due to the small globular shape and the intrinsic penetration enhancing effect of its components[253]. In another study, several formulations based on triacetin, Capryol 90, labrafil, Tween-80, Pluronic F127 as surfactants and PEG and transcutol as co-surfactants were tested as o/w, w/o and bi-continuous NEs for the transdermal delivery of indomethacin. Pluronic-based formulations possess the smallest particle size and the highest viscosity. An o/w and a w/o emulsion were selected as hydrophilic and hydrophobic formulations respectively for in vivo studies and a sustained systemic effect of 32 h was reported. Besides, a superiority of NEs compared to other formulations was reported in terms of solubilization of indomethacin and high plasma concentrations[237].

Another study focuses on the incorporation of saquinavir mesylate in self-emulsifying carriers containing clove oil, labrasol and transcutol (Figure 16.) that were loaded in polymeric poly (vinyl alcohol) (PVA)-transdermal films. These systems are self-nanoemulsifying drug delivery system-loaded polymeric transdermal films. By this 2-step formulation technique, an improvement of bioavailability due to the enhancement of drug's low solubility and improved skin permeation was observed compared to the oral dosage form, characterized also by an excellent patient compliance and reduced side-effects. Moreover, the AUC was found 2-times higher for the NEs loaded in the polymeric film, compared to the polymeric film containing only the drug without nanoemulsification[254].

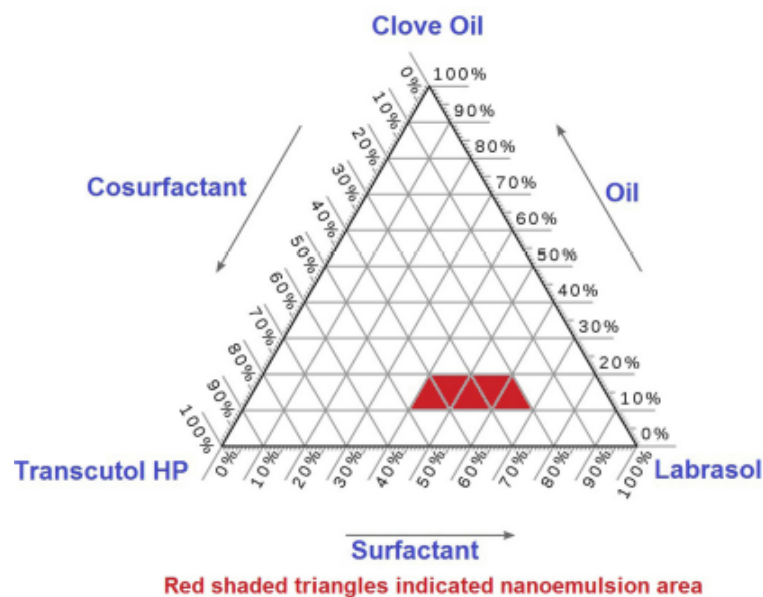**Red shaded triangles indicated nanoemulsion area**

Figure 16. Pseudo-ternary phase diagram for determination of SQR emulsification regions in selected SNEDDS components. **Abbreviations:** SQR, saquinavir, SNEDDS, self-nanoemulsifying drug delivery system (Adapted from [254]).

Hydrogel-based NE-system synthesized from isopropyl myristate, Tween-80 and Transcutol P was investigated for the transdermal delivery of fluvastatin with the use of Carbopol 940 as gelling agent. A higher skin penetration was found for the NE and the NE-gel compared to fluvastatin solution. More specifically, NE-gel exhibited better results, in terms of skin permeation being promising for the effective treatment of osteoporosis[255]. Asiaticoside was introduced in NEs where glycerol monooleate was used as oil, ethoxylated hydrogenated castor oil as surfactant and Transcutol P as cosurfactant. Gelling agent Carbomer 940 converted NEs into NE-gels for the treatment of skin scar. These formulations overcame the low water solubility and poor lipophilicity of the drug and an enhanced skin penetration, prolonged release and high bioavailability was achieved. The use of NEs as well as NEs carrying the API, as it can be observed from Figure 17., fluidizes the SC and provokes small changes in the skin microenvironment[256].
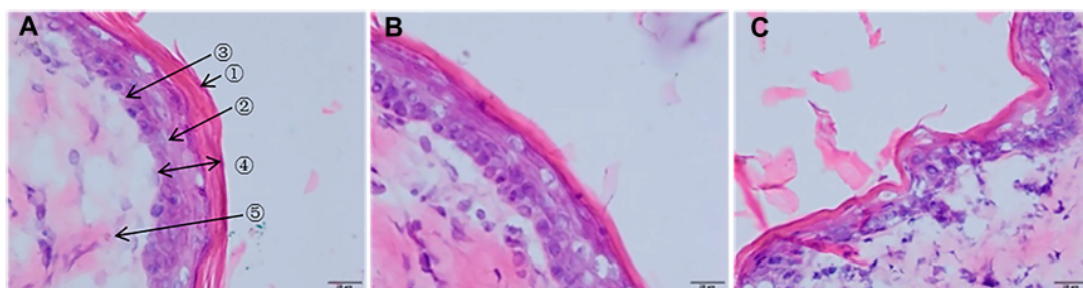
Figure 17. Histological photomicrographs of frozen section taken from rabbit treated with various applications (× 400). **Notes:** Saline (A); ASI-NEs (B); ASI-NBGs (C); ①stratum corneum; ②stratum spinosum; ③ stratum basale; ④ epidermis; ⑤dermis**. Abbreviations:** ASI-NEs, asiaticoside-loaded nanoemulsions; ASI-NBGs, asiaticoside-loaded nanoemulsions-based gels. (Adapted from [256]).

NEs synthesized from the cosurfactant isopropyl alcohol, the surfactant Brij30 and Brij35 and distilled water and they were tested as promising transdermal carriers for hydrophilic compounds. The skin permeation rate was improved and the lag time was shortened significantly. The NEs were tested for the model hydrophilic drug ropinirole hydrochloride and were found stable for 3 months[257]. Moreover, triptolide was incorporated in NE and NE-gels and compared with conventional gels in terms of effectiveness for the treatment of dermatitis. The bioavailability of the NE-based formulations was found significantly higher. These carriers, made of lipid Capryol 90, surfactant OP-10 and co-surfactant 1,2-propanediol, improve the skin penetration as they hydrate keratin and thus change the microstructure of SC resulting in higher percutaneous concentration of drug compared to other carriers[258].

4. INORGANIC NANOSYSTEMS

In the last part of this review several examples of inorganic nanoparticles will be discussed, emphasizing on their unique physicochemical properties which render them appropriate for transdermal applications. In general, there are contradicting results regarding their physicochemical parameters in the literature due to the complexity of the skin penetration mechanism that makes quite impossible the investigation of single parameters. Thus, the final effectiveness of these systems depends on the interplay of various physicochemical parameters along with the formulation, the environmental and the mechanical parameters[259].

4.1 Carbon-based Nanoparticles

Graphene is considered a 2-D material composed of sp^2 hybridized carbon atoms in a hexagonal arrangement. Graphene oxide (GO) and reduced graphene oxide (r-GO) are members of the graphene family[260,261]. GO was dispersed in macroporous PVA-films for the transdermal delivery of the anti-inflammatory drug ketoprofen. PVA was used as the polymer matrix due to its film-forming properties. The contribution of exfoliated GO in these vehicles was significant, as it slowed down the rate of diffusion and drug release through the change of the mechanical properties of the film (Figure 18.)[262].
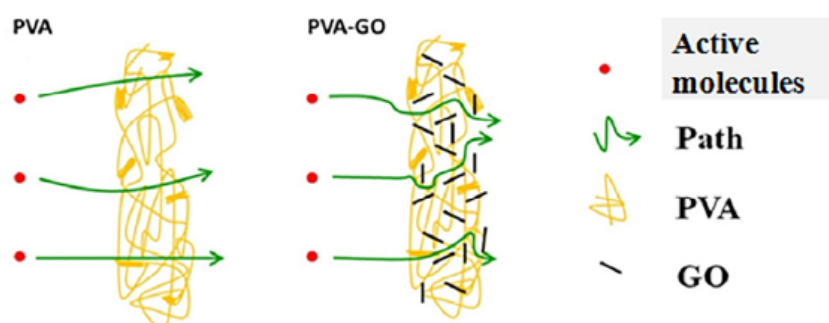

Figure 18. Schematic cartoons of modulated release of between neat poly (vinyl alcohol) (PVA) and PVA nanocomposite films. (Adapted from [262]).

Similarly, ondansetron loaded into Pluronic® F127 r-GO hydrogel exhibited a more sustained release compared to the hydrogel without GO and the oral solution. The retarding effect of GO in the drug release is attributed to the π-π stacking between the drug and the r-GO[263]. Another example is the Pluronic® F127 GO hydrogel for the transdermal delivery of tulobuterol showing a prolonged drug release for 72h, due to strong π-π interactions[264]. Lastly, Kapton flexible transdermal patch was used for the transdermal delivery of ondansetron triggered by local laser irradiation. GO was used as an NIR-absorbing agent for the improvement of drug's penetration ability. Remarkably, r-GO is not delivered in parallel with the drug and thus there is no concern regarding the safety of the proposed system[265].

Carbon nanotubes possess a cylindrical, hollow structure, of rolled up graphene sheet, and they are synthesized in the form of single-walled, double-walled, or multi-walled, depending on the number of graphene layers they are composed of[260,266]. Carbon nanotubes possess a large surface area which makes them promising for transdermal delivery of drugs due to their ability to absorb APIs and then release them via a depot-effect. They do not penetrate the skin but they may act as PEs in some cases. Also, apart from adsorptive materials, they are electroconductive which makes possible the application of iontophoresis for combination of both active and passive skin penetration techniques for hydrophilic and lipophilic drugs' penetration. However, the main disadvantage of this type of carriers is their low biocompatibility[267]. Multiwalled carbon nanotubes were used in hydrogels along with carboxymethyl guar gum for the delivery of the model drug diclofenac sodium transdermally. It was reported that with the addition of carbon nanotubes, the drug encapsulation was improved, and a prolonged release was achieved due to a higher viscosity compared to neat carboxymethyl guar gum. Among 0.5, 1 and 3 wt.% of carbon nanotubes, 1 wt.% was the optimal concentration dispersed in the hydrogel exhibiting the slowest release, attributed to the high viscous resistance[268]. Carbon nanotube-buckypaper were tested for the transdermal delivery of clonidine hydrochloride and flurbiprofen. The drug release profiles were affected by the type of carbon nanotubes (single-wall or multiwall), the functionalization with carboxyl or hydroxyl groups, the structure of the drug, the mass ratio carbon nanotube: drug and the number of carbon nanotube

layers (one or two) in the buckypaper. More specifically, the addition of a second layer of carbon nanotubes on the buckypaper led to a more prolonged drug release and reduced the initial burst effect. Also, multiwalled carbon nanotubes functionalized with carboxylic moieties exhibited better release profile for clonidine hydrochloride[269].

4.2 Metallic Nanoparticles

Plasmonic materials, which are composed of noble metals (mainly Au and Ag) exhibiting the surface plasmon resonance band due to the collective oscillations of free e$^-$, are very promising in biomedical applications. Generally, they show a good biocompatibility, enhanced stability and tunable synthesis and functionalization. Several parameters like size, shape, surface charge, core composition, water solubility and functionalization play a key role in their interaction with the skin[270]. It was reported that for silver nanoparticles (Ag NPs) the main mechanism may be the ion release through passive diffusion, however further studies are needed in order to conclude if Ag NPs are transported in deeper skin layers intact or as ions[271]. For gold nanoparticles (GNPs) the mechanism is also unclear. However, it was reported that they penetrate the skin in a higher amount compared to Ag NPs. Also, since Au ions were not found in physiological solutions, they are very promising as they are characterized by a higher biocompatibility and long-term stability than Ag NPs[272,273]. Regarding the effect of size, a comparison between 15, 102 and 108 nm citrate-capped GNPs has shown that the smaller the size, the higher the penetration and the diffusion coefficient[274]. In another study the parameter of shape was investigated via the comparison of PEG-capped Au nanorods and Au nanospheres and it was concluded that for both positively and negatively charged NPs, the best permeation ability was found for Au nanorods[275]. Also, Ag NPs of similar hydrodynamic diameter and zeta potential with spherical, triangular and rod shape were compared, and the highest penetration ability was reported for the rod-shaped ones[276]. The effect of surface charge and functionalization is also crucial for this type of carriers. For example, it was found that hydrophilic citrate-capped GNPs tend to accumulate on the superficial

layers of the SC, in contrast to hydrophobic dodecanethiol-capped GNPs, which penetrate the deeper skin layers[277]. Another combinatorial study regarding the size, hydrophilicity and vehicle properties of GNPs has shown that 6nm GNPs show more skin penetration than those of 15nm. Also, hydrophobic nanoparticles and more specifically dodecanethiol and cetrimide-capped GNPs, were characterized by a deeper skin penetration compared to lecithin and citrate-capped ones. Besides, citrate-capped GNPs didn't show skin penetration at all. Lastly, regarding the effect of toluene on lipid extraction from the SC, no drastic changes were observed, reporting thus a minimal effect of toluene in the SC compared to water[278]. Moreover, citrate (negatively charged), poly (vinyl pyrrolidone) (PVP) (neutral) and cetyltrimethylammonium bromide (CTAB) (positively charged)-capped GNPs were investigated for their skin penetration ability. The best skin penetration ability was found for the positively charged nanoparticles following the paracellular and transcellular route[279]. Lastly, GNPs functionalized with cell-penetrating peptides TAT and R_7 showed a deeper skin penetration compared to the PEGylated ones[275].

Some examples of use of GNPs, Ag NPs and iron nanoparticles (Fe NPs) in transdermal drug delivery will be also discussed. Vascular endothelial growth factor was conjugated with GNPs for its transdermal transportation. Negatively charged GNPs were found to possess better penetration profile after being compared with positively-charged and neutral ones. In the bio-conjugated nanoparticles, the charge was kept negative and thus an effective penetration in the epidermis was achieved. Also, it was reported that GNPs show the ability to protect the protein activity through binding[280]. A different approach was followed by Gupta et al., who studied through computational modelling the skin penetration of horseradish peroxidase with and without conjugation to GNPs. It was found that a free energy barrier exists for the protein skin penetration when the protein was used alone. In contrast, when the protein was conjugated to 3 nm GNPs, no barrier existed. GNPs modified the structure of SC by a softening effect and rendering easier the protein penetration[281]. A pectin-based Ag-nanocomposite film was synthesized incorporating Donepezil and exhibited an outstanding release efficiency. Ag NPs contributed in the prolonged release profile

of the drug as well as in the antimicrobial activity of the films, being thus promising transdermal carriers as they can avoid at the same time the contamination by sweat of the skin[282]. Lastly, superparamagnetic iron-oxide nanoparticles were used for the attachment of the chemotherapeutic agent epirubicin and its transportation transdermally. The mechanism of action of the above system is illustrated in Figure 19. These carriers exhibited a pH-responsive drug release in acidic microenvironment due to the pH-sensitive amide bond. By the external magnetic field, a deeper skin penetration was achieved, as the above carriers were able to penetrate the SC easier and through the follicular route[283].
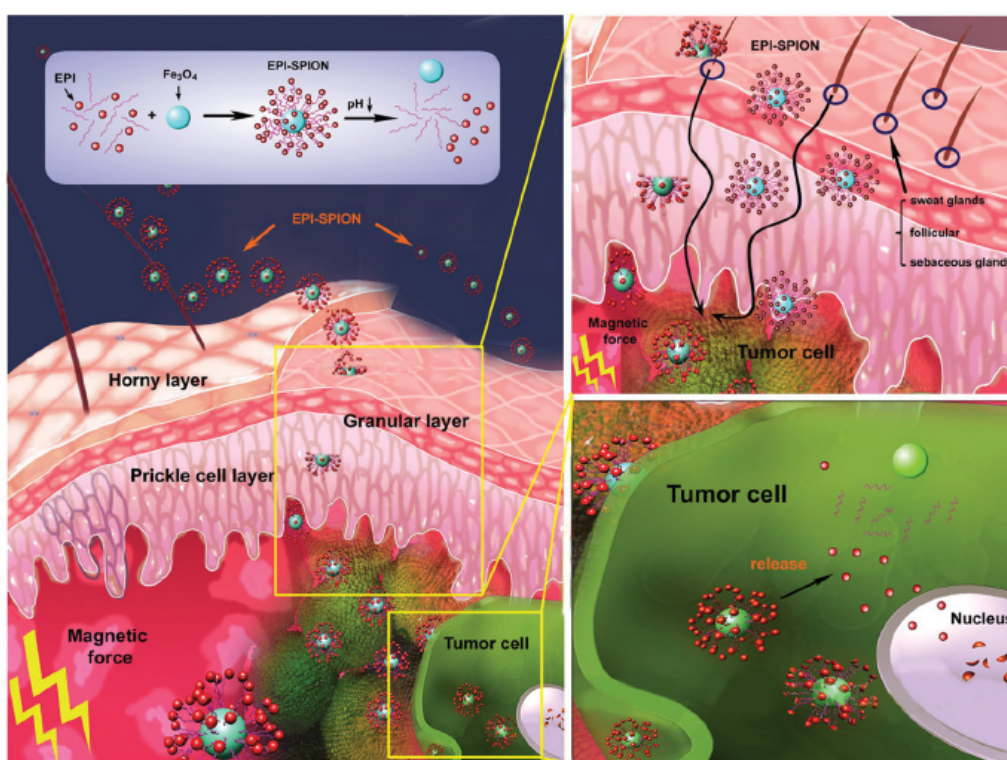
Figure 19. Schematic mechanism of EPI–SPION composites for magnetic transdermal delivery to combat skin cancer. The superfine pH-sensitive drug-loaded SPION was formed and transdermally delivered via a transfollicular path with the aid of an external magnetic force. The EPI was released inside the relatively acidic tumor tissues and then taken up by tumor cells. **Abbreviations:** EPI, epirubicin, SPION, superparamagnetic oxide nanoparticle (Adapted from [283]).

4.3 Quantum dots

Quantum dots (QDs), nanocrystals with core of a semiconductor material and a surface coating, are not used commonly for the transdermal administration of drugs. Due to their small size of few nanometers, QDs are strongly connected with toxicity effects as they can penetrate easily the healthy cells and accumulate in liver and kidney. Also, a major concern is the release of toxic ions (Cd^{+2}, Se^{-2}, Te^{-2}) during the degradation of their core[284]. However, in a report ZnO QDs grafted with the antagonist BQ-789 which binds to specific receptors of melanocytes were investigated in tyrosinase inhibition as transdermal carrier for ellagic acid. A good biocompatibility, enhanced targeting and skin permeation as well as controlled drug release were reported. Specifically, ellagic acid release was governed by the dissolution of ZnO QDs to Zn ions according to the pH gradient inside the cells[285]. The mechanism of action of the proposed system is represented below, in Figure 20.
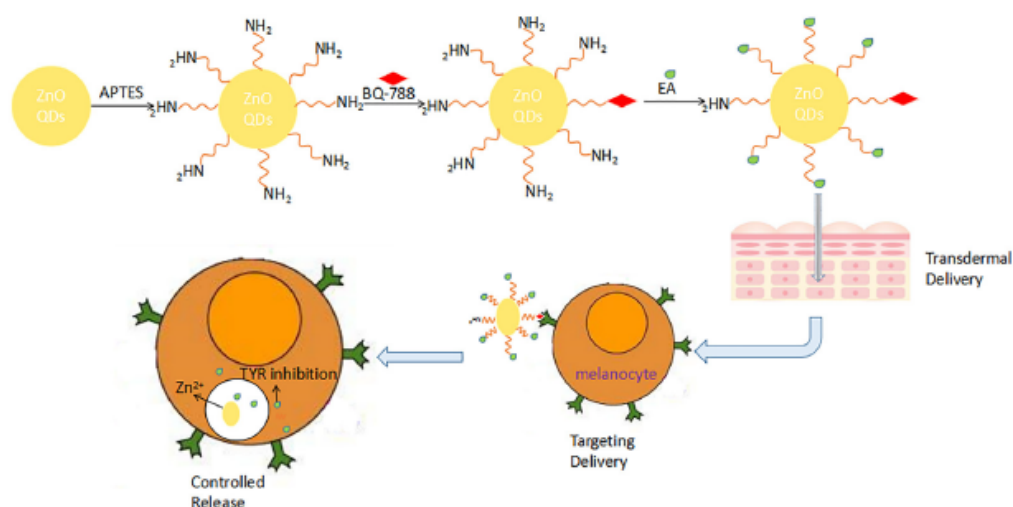
Figure 20. BQ-788/EA-ZnO was facilely synthesized with the aim of transdermal and targeting ellagic acid (EA) delivery as well as controlled EA release to melanocytes for tyrosine (TYR) activity and melanin deposition inhibition. (Adapted from [285]).

Generally, QDs can penetrate the SC due to their small size which is the driving force and depending on the other physicochemical properties, the mechanism of skin penetration may be slightly affected. Thus, a risk assessment should be applied when studies are investigating this type of NPs for skin applications. For example, 2 types of

QDs were compared, possessing a spherical and an ellipsoid shape as well as a positive, negative and neutral surface charge due to amine, carboxylic and PEG modification respectively. In all cases the penetration of SC was completed within 8h, except for the carboxylic-acid ellipsoid QDs that needed 24h to penetrate the SC[286].

4.4 Mesoporous silica Nanoparticles

Mesoporous silica nanoparticles (MSNs) are characterized by a high surface area and large pore volume, good stability, 2 functional surfaces for modification (internal and external) and sizes between 50 and 300 nm[287]. Amino functionalization of MSNs enhances drug encapsulation via hydrogen bonds or electrostatic interactions and improves the controlled release properties of the system[288,289]. Amino-functionalized MSNs were used for the encapsulation of hydrophilic 5-flurouracol and hydrophobic dexamethasone. A high loading capacity of both drugs was achieved as well as a sustained drug delivery and an enhanced skin permeability. The combination of both drugs was advantageous due to the synergistic effect and the ability to overpass the 5-flurouracol resistance induced by dexamethasone. Also, these carriers were found to enhance the antiproliferative effect of dexamethasone and showed enhanced targeting to melanoma cells[290]. The accumulation in different skin layers and receptor media when delivered by MSN-NH2 and free in gel are represented in Figure 21.
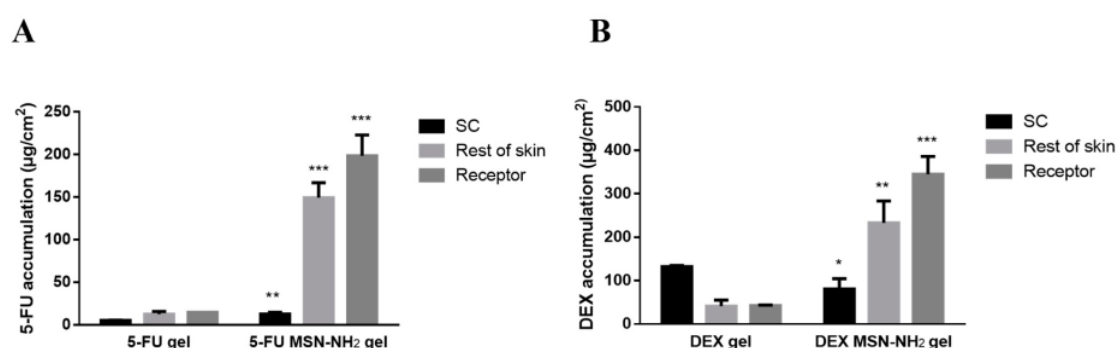

Figure 21. 5-FU **(A)** and DEX **(B)** accumulation in different skin layers and receptor media when delivered by MSN-NH2 and free in gel. Data are represented as mean _ SD (n = 3). Statistical significance was obtained with p-values _ 0.05, where * p _ 0.05, ** p _ 0.01, *** p _ 0.001. (Adapted from [290])

MSNs were also used for the transdermal delivery of small interfering RNA for skin cancer treatment with a good loading efficiency, stability and controlled release. Also, a poly-L-lysine (PLL) coating was added to MSNs, converting them from negatively-charged to positively-charged and thus improving significantly the cell internalization and the transdermal efficacy[291]. The methodology that was followed for the preparation of these carriers, is depicted in Figure 22.
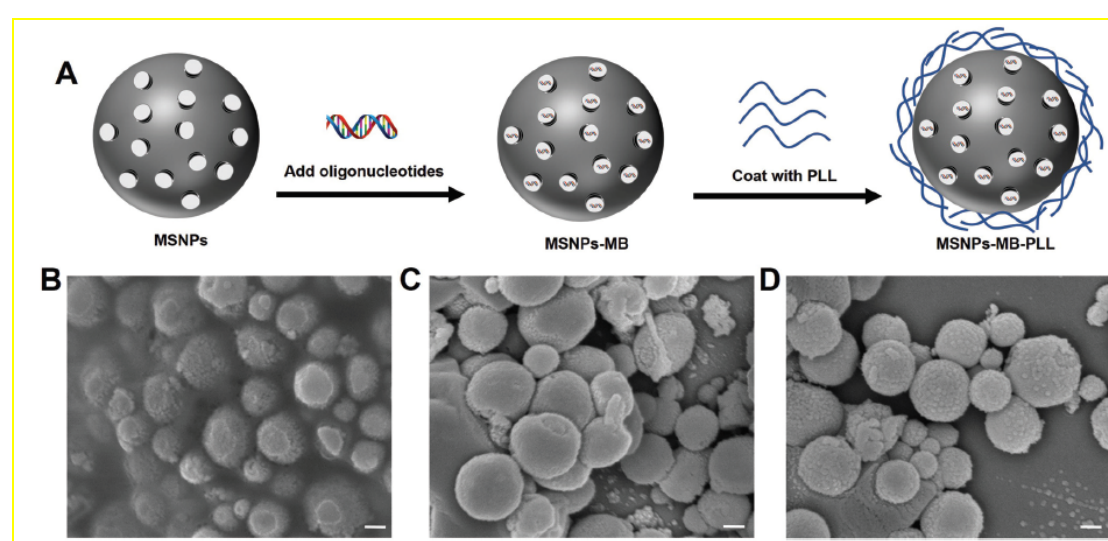
Figure 22. Preparation of MSNP-oligonucleotide complexes: **(A)** illustration of the synthesis of MSNPs, MSNPs-MB, and MSNPs-MB-PLL. Scanning electron microscopy images of **(B)** MSNPs, **(C)** MSNPs-MB and **(D)** MSNPs-MB-PLL. Scale bar: 100 nm. Abbreviations: MSNPs, mesoporous silica nanoparticles, MB, molecular beacon, PLL, poly-L-lysine (Adapted from [291]).

In another report lidocaine was entrapped in MSNs for the improvement of its low bioavailability and for a faster drug release. MSNs that were modified with positively charged amino-propyl groups enhanced lidocaine's skin penetration due to the electrostatic interactions between the carriers and the negatively-charged membranes[292]. MSNs encapsulated methotrexate in order to enhance the epidermal accumulation of the drug overpassing its limitation of low lipophilicity. Also, shea butter was used as a PE and as a result, a deeper penetration of MSN in the skin was found[293].

In Figure 23., an Ishikawa diagram represents the key parameters for the design and the development of a transdermal delivery platform.
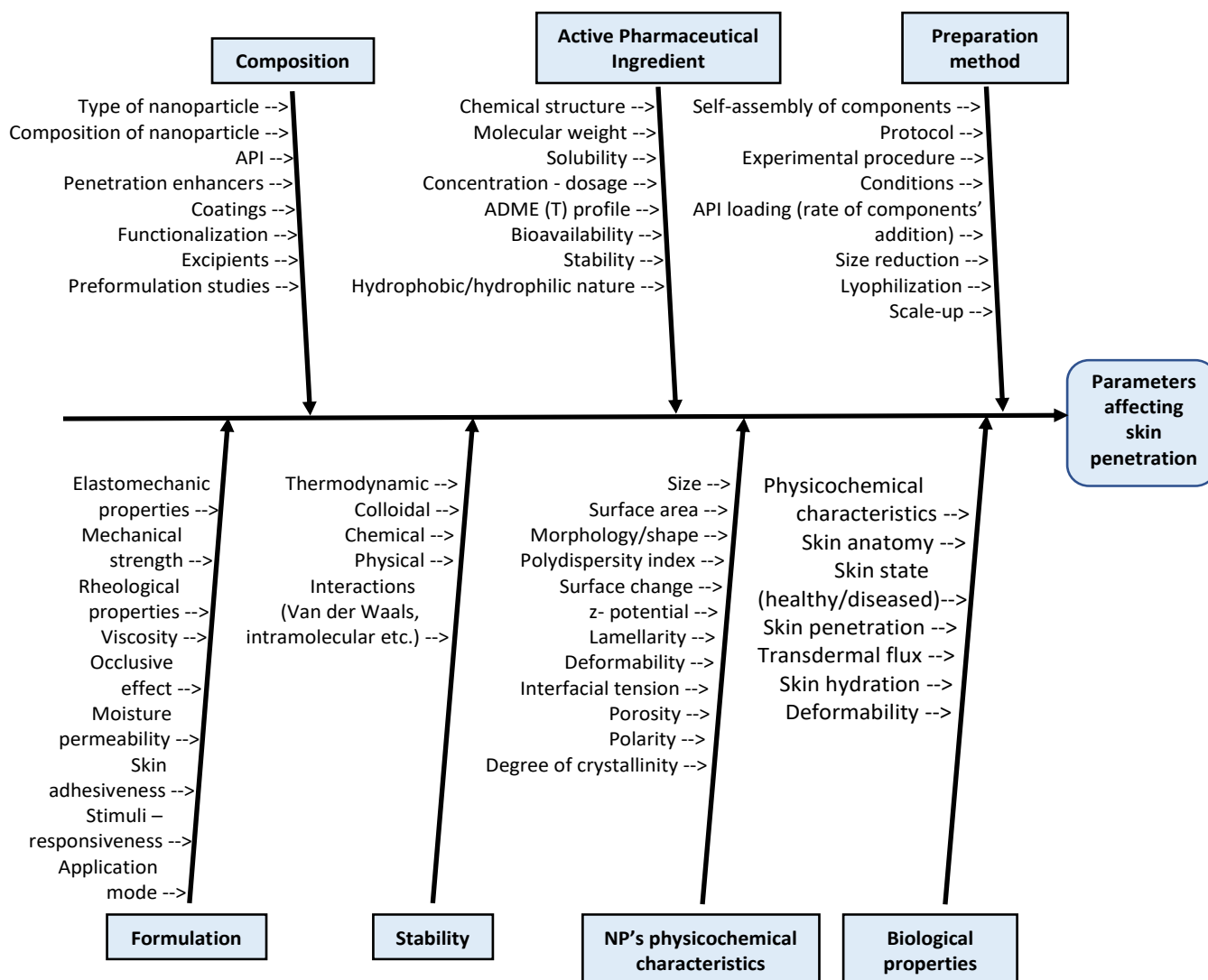
Figure 23. Ishikawa diagram presenting the parameters that affect the skin penetration in transdermal drug delivery systems. **Abbreviations:** API, active pharmaceutical ingredient, ADME(T), absorption-distribution-metabolism-elimination-(toxicity), NP, nanoparticle

Finally, in Table 1, some indicative examples of transdermal drug delivery systems are presented, emphasizing on the nanoparticle that is being used, the API which is carried and the unique physicochemical properties of each system.

Table 1: Overview of different types of nanoparticles used in transdermal drug delivery. The active substance that these systems transport and their physicochemical properties are presented below.

Publication	Nanoparticle(s) (NPs)	Active Pharmaceutical Ingredient (API)	Physicochemical characteristics			
			Size (nm)	Polydispersity index (PDI)	z-potential (mV)	Entrapment efficiency (EE%)
Park et al. [83]	Liposomes	Resveratrol	279.85	n.m.	+26.5	82.95
Shi et al. [73]	Liposomes	Salvianolic acid B	183.2 ± 4.09	0.190	-9.25 ± 0.92	86.70 ± 0.85
Knudsen et al. [90]	Liposomes	Calcipotriol	93.5 ± 1.8	0.044 ± 0.005	-8.4 ± 0.2	n.m.
Shreya et al. [116]	Transferosomes	Asenapine maleate	126	0.232	-43.7	54.96
Ma et al. [111]	Ethosomes	Paeonol	120.2 ± 1.3	0.131± 0.006	-16.8 ± 0.36	84.33 ± 1.34
Paliwal et al. [106]	Ethosomes	Flurbiprofen	162.2 ± 2	0.341	-48.14 ± 1.4	92.1 ± 1
Song et al. [146]	Transethosomes	Sinomenine hydrochloride	93.2 ± 7.2	0.168 ± 0.023	-17.6 ± 3.6	59.9 ± 4.5
Albash et al. [147]	Transethosomes	Olmesartan medoxomil	222.60 ± 2.50	0.11 ± 0.06	-20.80 ± 0.30	58.50 ± 1.30
Qadri et al. [123]	Invasomes	Isradipine	194 ± 18	0.272 ± 0.062	n.m.	88.46 ± 9.26
Tawfik et al. [126]	Invasomes	Agomelatine	313 ± 12	0.14 − 0.65	-64 ± 4.1	78.6 ± 3.1
El Ridy et al. [132]	Niosomes	Lornoxicam	295.3	0.391	-46.9 ± 9.83	66.11 ± 2.50
Zhang et al. [135]	Niosomes	Salidroside	232.9 ± 9.7	n.m.	-45.3 ± 5.8	33.74 ± 0.57
Wen et al. [141]	Proniosomes	Mefenamic acid	408.2 ± 89.3	0.737	-42.9 ± 7.27	82.84 ± 10.8
Albash et al. [142]	Bilosomes	Olmesartan medoxomil	559.30 ± 10.70	0.57 ± 0.15	-38.35 ± 0.65	72.49 ± 0.38
Zhang et al. [151]	Glycerosomes	Paeoniflorin	198.75 ± 15.93	0.24 ± 0.02	-16.40 ± 1.20	51.24 ± 1.79
Zhang et al. [149]	Flavosomes	Quercetin	99.33 ± 1.46	0.261 ± 0.003	+21.4 ± 0.7	90.37 ± 1.31
Guo et al. [162]	Solid lipid nanoparticles	Ivermectin	312.8 ± 2.40	0.082 ± 0.005	-30.5 ± 1.51	98.48 ± 0.052
Mendes et al. [171]	Nanostructured lipid carriers	Donepezil	177.05 ± 2.12	0.25 ± 0.02	-55.35 ± 2.28	99.4 ± 0.03
Alam et al. [170]	Nanostructured lipid carriers	Pioglitazone	166.05	0.18	-27.5	84.56
Yang et al. [246]	Nanoemulsion	Sulconazole	52.3 ± 3.8	0.205 ± 0.02	+23.2 ± 1.2	87.1 ± 3.2
Zhou et al. [195]	MPEG-PCL micelles	Curcumin	48.75 ± 1.90	n.m.	-13.2 ± 0.8	93.57 ± 1.67
Bahadoran et al. [205]	PAMAM dendrimers	pIRES-H5/GFP DNA plasmid	105-115	n.m.	+42-45	n.m.
Abnoos et al. [222]	Chitosan-Alginate nanoparticles	Pirfenidone	80	0.020 ± 0.008	n.m.	94.08
Vijayan et al. [228]	PLA nanoparticles	Repaglinide	108.6 ± 3.4	0.06	-17.61 ± 3.42	92.7 ± 1.4
Nafisi et al. [292]	Mesoporous silica nanoparticles	Lidocaine	95	n.m.	+33	99.73
Luo et al. [264]	Reduced graphene oxide hydrogel	Tulobuterol	271	0.013	n.m.	98.25 - 99.28

Abbreviations: GFP, green fluorescent protein, PAMAM, poly (amidoamine), NPs, nanoparticles, n.m., not mentioned, API, active pharmaceutical ingredient, PDI, polydispersity index, PLA, poly (lactic acid), EE, entrapment/encapsulation efficiency, DNA, deoxyribonucleic acid, MPEG, methoxy-poly (ethylene glycol), PCL, poly (caprolactone)

E. CONCLUSIONS - FUTURE PERSPECTIVES

SC constitutes the skin's outermost layer that acts as a protective barrier. Transdermal drug delivery systems aim on the disruption of the SC cohesion and as a result the deeper skin penetration of API's and finally their systemic administration. This type of drug delivery systems offers many advantages as the patient compliance, the avoidance of first-pass metabolism effect, the sustained release of API's, the reduction of side effects, and the increase of API's bioavailability. On the other hand, the complexity of skin physiology and skin penetration mechanisms renders challenging the investigation of individual parameters that affect the transdermal administration of API's. NPs seem very promising in transdermal drug delivery systems, as due to their small size they can permeate easily the skin, or they can act as penetration enhancers by fluidizing the SC. Also, they are beneficial for the transportation of both hydrophilic and hydrophobic API's, and they offer a significant prolonged release effect.

Regarding the physicochemical parameters, the size, shape, surface charge, lamellarity, crystallinity and deformability are some of the key parameters which are still under investigation. Generally, smaller NPs can permeate the skin more easily. A large surface area also contributes to a direct contact with the skin, increasing the possibility of permeation. Regarding the role of charge, it is suggested that positively charged NPs can interact with the cell membrane. However, after they adhere on the skin, a negative surface charge may be helpful for their penetration in deeper layers due to the repulsive interactions with the membrane. Additives or coatings are very important as for example chitosan can invert a negative z-potential to a positive one and facilitate the interaction with the cell membrane, as well as PEGylation can increase the hydration of the SC and thus the expansion of the intercellular channels. Lamellarity is another significant parameter and MLVs seem very promising in transdermal administration due to a step-by-step loss of bilayers during the process of skin penetration. Furthermore, the elasticity has a crucial role, as the deformable properties can allow the NP to squeeze through microscopic channels and reach the deeper layers. Hydrophobicity or hydrophilicity, crystallinity and thermodynamic state of NPs are also important and should be taken into consideration. Formulation

characteristics as viscosity, skin adhesiveness, elastomechanic properties, moisture permeability and application mode also affect the design of an effective transdermal drug delivery system. Remarkably, the stability of these systems (thermodynamic, colloidal, chemical, and physical) is a prerequisite for a safe and effective formulation. Moreover, the components of the transdermal systems as well as the preparation method that has been followed influences its properties. On the one hand, the chemical structure, solubility, bioavailability, polarity, and concentration of the API should be considered. On the other hand, the type and concentration of NPs, the functionalization degree and finally the interaction between the API and the NPs are key factors. The role of PEs which either alter the lipid conformation of the SC or disrupt the cohesion of corneocytes, is also of high importance.

Comparing the types of NPs, lipid-based NPs which either penetrate intact the SC, or fuse with this and release the drug or act as penetration enhancers, seem the most promising due to their similarity to SC. More specifically, deformable liposomes can achieve a deeper skin penetration compared to conventional liposomes. Also, polymer-based NPs offer a better stability than lipid-based systems, possibilities for stimuli-responsiveness and due to their smaller size, they easily penetrate the skin as well as they follow the follicular pathway acting as drug reservoirs. Regarding NEs which are characterized by a good colloidal stability, most reports are focusing on o/w NEs and in the formulation of gels. Lastly, the reports for inorganic NPs are limited due to issues regarding their safety and biocompatibility.

As transdermal drug delivery systems target the disruption of the SC structure and the reach of systemic circulation, a very careful risk-assessment strategy of these formulations should be performed to avoid nano-toxicity concerns. The most important is the restoration of the SC structure and the return to its normal protective function to avoid serious side-effects. Also, apart from the safety of these systems, their complexity and reproducibility as well as the cost-effectiveness, the challenging extrapolation of in vitro and animal's experimental data to humans and the unclear regulatory landscape of nano-formulations should be considered for an effective clinical translation.

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